TECHNICAL REPORT
FD-11

OXIDATION CHANGES AFFECTING ODOR AND FLAVOR OF FREEZE-DRIED MEATS

by

J. R. Chipault J. M. Hawkins

UNIVERSITY OF MINNESOTA
(HORMEL INSTITUTE)
Austin, Minnesota

Contract No. DA 19-129-QM-1994

May 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

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FOREWORD

Oxidative changes resulting from contact with atmospheric oxygen have long been recognized as a major cause of deterioration and loss of acceptability in foods of both animal and vegetable origin. Experience has emphasized the significance of such changes during the storage of freeze dried foods as prepared for military rations. An earlier study, contract DA19-129-QM-1725, titled: Factors Controlling Rancidity Development in Freeze-Dried Meats, revealed that in addition to the oxidative changes leading to the rancidity of neutral fats, there is an immediate, non-autocatalytic absorption of gaseous oxygen by freeze dried meat which is associated with the development of off-odors, sometimes identified as stale or putrid. This contract seeks to extend our knowledge of the nature and significance of these latter oxidative changes and to identify the effect of raw materials, processing factors and other variables which may be exploited for their control.

The study herewith reported was conducted by the Hormel Institute of the University of Minnesota under contract DA19-129-QM-1994. Dr. J. R. Chipault, who served as official investigator, was assisted by Mr. J. M. Hawkins. The project officer for the U. S. Army Natick Laboratories was Dr. Maxwell C. Brockmann of the Animal Products Branch. Alternate project officer was Mr. Albert S. Henick of the Food Chemistry Branch.

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OXIDATION CHANGES AFFECTING ODOR AND FLAVOR OF FREEZE-DRIED MEATS

SUMMARY

Samples of commercially-prepared freeze-dried cooked and raw beef, raw pork, raw fish and shrimp have been examined in a Warburg apparatus and their oxygen absorption characteristics have been measured. The moisture content, glyceride fat and bound lipids have been determined also. The oxidation of these materials appears to be complex. It is a two-step process involving first oxidation of the bound lipids, followed, after a variable period of lower oxygen absorption, by oxidation of the glyceride fat. The rates of oxidation during these two steps and the length of the period of low oxygen absorption between them are determined by the nature and history of the sample.

In these commercially prepared samples of unknown history no correlation could be established between absorption of oxygen and type of product, moisture content, bound lipids or glyceride fat content.

Large variations in the over-all rates of oxygen absorption were observed between different lots of the same type of material, between different containers of the same lot and even between different portions of product taken from the same container.

The oxidation of freeze-dried raw tissue appears to be due to autoxidation rather than to enzymatic action.

Samples of raw and cooked freeze-dried beef were prepared in the laboratory. They were fractionated in an atmosphere of pure nitrogen so that the fractions were not exposed to oxygen prior to oxidation studies.

The bound lipids absorb oxygen rapidly, with no induction period and no peroxides are formed. In contrast the isolated glyceride fat is much more resistant to autoxidation and shows a long induction period.

Antioxidants added to freeze-dried beef decreases slightly the rate of oxygen absorption by the bound lipids but their effect is small and they do not produce a true induction period.

Off-odors which develop rapidly in freeze-dried meat exposed to oxygen are not due to true rancidity of the glyceride fat. They appear to be due to the oxidative deterioration of the bound lipids and to the

formation of stale, putrid-like odors by water-soluble, non-lipid components of the crude bound lipids. This non-lipid fraction develops strong odors on warming but does not absorb oxygen.

Cooking has a tendency to increase organoleptic deterioration and oxygen absorption. Malonaldehyde accompanies odor formation in cooked but not in raw beef.

There is no difference in the rate of deterioration of two different muscles obtained from the same animal.

Moisture appears to be the most important single factor affecting autoxidation of freeze-dried beef. A moisture level of 1% or less is most desirable.

I. INTRODUCTION

The purpose of this research was to study the role played by lipids as a factor affecting the quality of freeze-dried meats and fish.

While freeze drying is a convenient means of preserving food, most dried meat and fish products deteriorate rapidly when exposed to oxygen. Undesirable odors and flavors develop rapidly and changes in texture associated with lower rehydratability also occur. Although the odor and flavor changes observed are not of a typical rancid nature, they are apparently associated with autoxidation of some of the lipids present in the dehydrated tissues. However, the exact nature of the changes involved and of the compounds responsible for these deteriorations are not known.

In this work a number of commercially prepared samples of freeze-dried meat and fish have been surveyed in an attempt to correlate the oxidative and organoleptic changes with gross characteristics of the materials, such as type of products, method of preparation, composition and storage.

Most significant results, however, were obtained with fresh samples prepared experimentally in our laboratory under rigidly controlled conditions.

II. SURVEY OF COMMERCIALLY PREPARED FREEZE-DRIED SAMPLES

A. Starting materials

Thirteen samples of cooked beef, nine samples of raw beef, two samples of raw pork, three samples of raw fish and one sample of raw shrimp were furnished by the Quartermaster Food and Container Institute for this study. A description of the samples and other information furnished with them have been recorded in Table I. These products had been prepared several years before the beginning of this study and had been packed, presumably under vacuum or in an inert atmosphere, in sealed cans and were kept at room temperature until used. However, details regarding the nature of the fresh tissue processing methods and storage conditions are unknown.

B. Experimental procedures

1. General

After opening a container, the contents, if they consisted of slices or large pieces, were broken up manually into particles of approximately the same size as those of dehydrated ground beef. These were then mixed thoroughly by hand to give a homogeneous lot of material from which representative samples were removed for further investigation

2. Moisture determination

The moisture content of the various samples was determined by drying a portion of the material in a vacuum oven at 102°C under pressure of 15 mm of mercury for 24 hours.

3. Lipid extractions

Portions of the samples were extracted in a soxhlet apparatus with low-boiling petroleum ether for 14 hours. The extracts were freed of solvent in a vacuum rotating evaporator and the amount of fat extracted was determined gravimetrically. This lipid material extractable with petroleum ether, represents loosely bound free fat consisting mostly of glycerides, and will be referred to as glyceride fat.

A portion of the petroleum ether extracted tissue was further extracted with a mixture of equal volumes of chloroform and methanol in a Waring blendor for 2 minutes under nitrogen. The mixture was filtered with suction on a Büchner funnel and the extraction was repeated twice more on the residue using a total volume of solvent equivalent to seven times the weight of the extracted tissue. Solvents were removed from the extract and from the residual tissue under vacuum. The lipids extracted with chloroform:methanol contained mostly phospholipids, proteolipids and other complex lipid material which are held tightly by the protein of the tissue by physical or chemical forces are not extractable by non-polar lipid solvents. These lipids have been designated bound lipids, while the residual tissue has been labeled fat-free tissue.

4. Oxygen absorption measurements

Samples of 0.5 to 2 grams of whole tissue, petroleum ether extracted tissue, glyceride fat, bound lipids or fat-free tissue were introduced into calibrated Warburg flasks. The flasks were flushed with pure oxygen and placed in constant temperature baths at 40 or 60°. After an equilabration period of 15 minutes, the manometers were closed and measurements of oxygen absorption began.

C. Results

1. Composition of freeze-dried sample

The moisture, glyceride fat and bound lipid content of the samples studied are shown in Table II. In general, the moisture content of the samples was quite low. Of the 28 samples received, 24 contained 2% or less moisture, and of these, 10 contained less than 1% of water. In general, the cooked products with a moisture range between 0.3 and 1.8% were dryer than the raw samples which ranged from 0.4 for a sample of pork chops to 3.1 for fish squares.

The fat content of the beef samples varied between 20 and 50%, the two pork samples contained close to 50% lipids, while the fish and shrimp contained less than 15% of this component. There appears to be a

correlation between the glyceride fat and bound lipid contents of the various samples and each of these fractions varies independently from the other. The lipids of the meat consist mostly of free glyceride fat extractable with petroleum ether, while the marine products contain very little free fat and more than 80% of the lipids are made up of bound lipids.

2. Oxygen absorption by cooked freeze-dried meats

Curves showing the over-all oxygen absorption by the cooked samples are shown in Figure 1. In order to obtain an over-all picture of the oxidation process, these samples were allowed to absorb oxygen to very high level for long periods of time. As they appear in Fig. 1, all the curves are S shaped and seem typical of the usual autocatalytic oxygen absorption pattern shown by most natural fats. The first stage, or induction period, is characterized by the absorption of a small but definite amount of oxygen. This is followed by a rapid oxygen absorption stage and the reaction terminates in a plateau with very little oxygen being absorbed, apparently when most of the oxidizable material has reacted with oxygen. All the samples examined however, showed a rapid initial absorption of oxygen which was of short duration. This oxygen absorption pattern is not shown by common natural fats and is not evident in Fig. 1 because of the scale to which this figure is drawn. This initial absorption of oxygen, however, is shown in Fig. 2 on an expanded scale for several typical compounds. Others have been omitted for sake of clarity.

The over-all oxygen absorption pattern, therefore, appears to consist of 4 stages: stage 1 is a rapid but short initial absorption of oxygen; stage 2 is a period of low absorption of oxygen similar to an induction period; stage 3 appears when a rapid autocalytic absorption of oxygen begins and terminates with the appearance of stage 4 when oxidation again decreases when all easily autoxidizable material has reacted.

Several samples such as L-29711 and A-E2107 show a very short stage 2 (induction period previous to oxidation of free glyceride fat) and one sample, W-2-3/60, has an oxygen absorption curve which appears to consist only of an initial rapid oxygen absorption followed by a long induction period. However, in all the samples examined, as well as in freshly prepared freeze-dried cooked meats kept in an atmosphere of nitrogen until studied, the initial rapid absorption of oxygen remained low and did not exceed levels of approximately 20 to 30 mmole of oxygen per kilo of fat. In contrast, sample W-2-3/60 has attained an oxygen absorption level of more than 150 mmoles per kilo of fat. As will be shown later, the initial oxygen uptake by the whole tissue samples is due to the oxidation of the bound lipids, and this oxidation level remains low because the concentration of bound lipids in the tissue is low. It is very possible, therefore, that the oxidation curve for sample W-2-3/60 does not show the initial oxidation stages but represents rather the last two stages of the total oxidation sequence, the previous stages having occurred before the sample was studied, probably during its preparation and storage.

The over-all shape of the oxygen absorption curves, the rates of oxygen uptake during the two active oxidation periods and the lengths of the intermediate phase during which little oxygen is absorbed will depend primarily on the composition of the lipids of the tissue, on the presence or absence of antioxidant or prooxidants and on the treatment received by the samples prior to study. These, in turn, are dependent on numerous variables related to the kind of animal from which the tissue was obtained, its age, health and nutritional state, the kind of tissue studied and the conditions prevailing before, during and after freeze drying. Since these commercial samples are prepared in large quantities using muscle tissue from different animals, and are cooked, freeze-dried and stored under different conditions, it is not surprising that the curves for these samples show no regular pattern which can be related to the particular type of tissue studied.

Figures 3, 4 and 5 show that curves which agree closely are obtained when duplicate portions of a small homogeneous sample are studied (Fig. 3), but that wide differences result when different cans from the same lot of material are used, or even when different samples are taken from the same can (Fig. 4) and that even more disagreements are apparent when different lots of the same type of material are studied (Fig. 5). In Figure 6 the oxygen absorption of several fractions obtained from samples W-1-2/60 has been plotted and expressed in terms of mmoles of oxygen absorbed per kilogram of bound lipids. The petroleum ether extracted tissue is essentially free of glyceride fat but still contains all the bound lipids. The curves for the bound lipids, the petroleum ether extracted tissue and the whole tissue are very similar, suggesting strongly that the oxygen uptake during this stage is due to absorption of oxygen by the same fraction, namely the bound lipids. The oxidation of the isolated petroleum ether soluble glyceride fat is not shown in this figure since this fraction had a long induction period of more than 600 hours before it began absorbing oxygen, and the subsequent oxygen absorption curve was typical of the usual autocatalytic autoxidation of ordinary fats.

The "fat-free tissue" from which both the petroleum ether soluble and methanol:chloroform soluble lipids had been removed absorbed small amounts of oxygen at a constant rate. In this case, the amount of oxygen absorbed by this fraction is expressed in terms of fat-free tissue and must be due to either residual lipid or to absorption by the protein components. However, further extraction of this fraction with chloroform:methanol yielded no additional lipid and had no effect on the absorption of oxygen by this sample. Consequently, if any lipids are left in this material, they must be different from the bound lipids extracted with the chloroform:methanol solvent.

Figures 7 and 8 show similar data for two other cooked beef samples. In both cases, the absorption of oxygen by the isolated bound lipids and by the tissue freed of glyceride fat by petroleum ether extraction are in good agreement. But in one case, the whole tissue absorbs less oxygen, and in the other case, considerably more oxygen than would be expected from the rate of oxidation of the isolated bound lipids. In

view of the large differences observed when different portions of the same lot of material were examined (Fig. 4) and since the material used for the study of oxidation by the whole tissue and that extracted to remove the lipid fractions were not part of the same homogeneous sample, no great significance is attached to these differences.

3. Oxygen absorption by raw freeze-dried meat and fish

Figure 9 shows the oxygen absorption of the raw freeze-dried meats. These materials were studied at 40°C. to prevent possible heat damage to these uncooked samples and enzyme destruction, although no enzymic activity was expected in materials with such a low moisture content. In general, the pattern of oxidation of the raw material is similar to that of the cooked product, except that with most samples and at the lower temperature employed, the stage of active oxygen uptake by the glyceride fat was not reached even though the experiments were extended for up to 8,000 hours. This stage however, was reached with the two pork chop samples and two samples of beef.

In Figure 9 the oxygen absorbed by the three samples of fish and the one shrimp sample has been plotted versus time. The shape of the curves corresponds to the absorption of oxygen by the bound lipids. These marine tissues, however, contain mostly bound lipids and very little glyceride fat so that the amount of oxygen absorbed when expressed in terms of total fat is considerably higher than for the meats which contain only small amounts of bound lipids. In these cases either the absorption of oxygen by the free glyceride fat has not been reached or the amount is so small that it is not noticeable when superimposed over the large amount of oxygen absorbed by the bound lipids.

In Figure 11 the oxygen absorption of a sample of raw fish has been studied at 40° and at 60°C. At the higher temperature, oxidation is much more rapid than at the lower temperature and the temperature effect indicates that the oxidation is purely a chemical reaction rather than due to an oxidative enzyme. In the latter case, the enzyme would be destroyed at 60°C. and, therefore, the rate of oxygen absorption would be lower than at 40°C.

III. LABORATORY SAMPLES

It was felt that the general history of the commercial samples was largely unknown and that this included factors such as source and nature of the starting material, time and temperature of storage before and after dehydration and previous exposure to oxygen which would affect the oxidative behavior of the samples. These uncertainties and lack of information make a study of the oxygen absorption of the samples difficult and unreliable. Consequently, further work was done with materials prepared in the laboratory under carefully controlled conditions and in an ambient atmosphere essentially free of oxygen.

A. Starting materials

The meat used for these studies has been mostly rib eye (longissimus dorsi) from U. S. Good rib roast obtained directly from

Geo. A. Hormel & Company. In one instance, it was desired to compare an unworking muscle with a working muscle and roasts from the rib eye and from the eye of the round were obtained from the same animal and studied in parallel experiments.

B. Experimental procedures

1. Preparation of samples

a) Trimming, cutting and grinding. The fresh meat was procured especially for us from animals aged in a packing house cool for two to three days after slaughter. Fatty and connective tissues surrounding the muscles or penetrating into it as large veins were manually dissected and only the lean tissue was used. The pieces of beef were further cooled to near freezing temperature for several hr. and either cut into two-inch cubes or ground, depending on the studies planned.

b) Cooking. When a cooked product was desired, the cubes were spread in a single layer on a nylon mesh woven approximately 1/4 inch above the bottom of an aluminum tray. A thermo-couple was inserted in the center of one of the cubes and the tray was placed in an autoclave where the meat was cooked with steam at atmospheric pressure until the thermo-couple registered a temperature of 70°C. The cubes were then removed, quickly weighed and frozen at -20°C., prior to freeze drying.

c) Freeze drying. In a first experiment, when it was desired to fractionate the freeze-dried meat by solvent extraction, slices of raw meat were frozen on a stainless steel screen held approximately 1/4 inch above the bottom of a metal can, 10 cm in diameter and 4 cm high, with a tightly fitting cover (originally containers for 100-foot spools of 35 mm film). A Virtis freeze drying apparatus consisting of tiered shelves for bulk drying, a clear plastic bulk drying drum and a cover were used for freeze drying the samples. A rack on which 4 cans containing the frozen meat slices could be accurately positioned was placed on the top shelf of the dryer. The cover was modified by drilling four holes through which were inserted 1/4 inch rods through a vacuum tight joint made from a piece of pressure rubber tubing. The can covers were attached at the end of the rods and by properly positioning the drying chamber cover, they were suspended, within the drying chamber, 1 to 2 cm above the cans containing the frozen meat slices. The samples were freeze-dried for 41 hours at a ~~pressure less than 0.1 mm of mercury with no additional heat supplied~~ other than the normal radiations absorbed by the samples from the ambient surroundings.

In order to prevent exposure of the dried material to oxygen during transfer from the freeze drying apparatus to the extraction chamber at the end of the freeze drying period, the covers were lowered onto the sample cans and then the vacuum was released with pure nitrogen. The cans were quickly removed and weighed and then introduced into the nitrogen atmosphere of the extraction chamber.

In other experiments where more material was needed, cubes of meat were frozen on nylon mesh suspended in 10-inch diameter pans. Six pans were stacked in the freeze drying drum with spacers between the pans to provide adequate free space for unrestricted passage of the water vapor from the drying chamber to the condensing unit. A thermistor was inserted in the center of one of the cubes and freeze drying was continued for three to five hours after the thermistor indicated that the meat had attained room temperature. After drying, the vacuum was released with pure nitrogen but the freeze-dried meats were exposed briefly to air for a few minutes while they were transferred from their freeze drying chamber into containers where they were again evacuated and flushed with nitrogen several times before they were stored in a nitrogen atmosphere at -20°C. until needed for further experiments. In all cases, the meat was used within 48 hours after it had been prepared. In a series of experiments where ground meat was used, 10 to 15 grams of ground tissue was pressed in a thin layer at the bottom of a 1 by 6 inch test tube and frozen at -20°C. The tubes were connected by means of large diameter rubber tubing to a vacuum manifold where the samples were freeze-dried at a pressure less than 0.1 mm of mercury. At the end of the drying period, each tube was removed individually from the manifold without disturbing the vacuum either in the tube or in the manifold. The tubes then could be connected to other pieces of apparatus for further treatment.

d) Extraction chamber. For fractionation of freeze-dried meat without exposure to oxygen, it was felt that a chamber in which the necessary manipulations could be performed in an atmosphere of pure nitrogen would be necessary. Such a chamber 40 inches wide, 28 inches deep and 36 inches high was constructed. The chamber was provided with six access ports fitted with rubber gloves, with observation windows on all four sides, and was equipped with the necessary electrical outlets and several tubing connections for introduction of water, vacuum lines and various gases. The chamber was also equipped with a large panel which can be removed to permit the introduction and installation of equipment prior to the beginning of an experiment. After the equipment has been properly installed and connected, this panel is replaced and sealed and the chamber is alternately flushed with carbon dioxide and pure nitrogen several times and finally left under a slight flow of nitrogen.

Small pieces of glassware or experimental samples are introduced into or removed from the chamber through a vacuum lock installed at one end of the box. This lock made of plexiglass is 4.75 inches square by 8 inches long inside. It has 4.75 inch square openings into the chamber and into the outside room, and when sealed is vacuum tight. With the chamber opening closed, materials may be placed into the lock and after closing the outside opening the lock can be thoroughly evacuated to a pressure less than 1 mm and flushed several times with pure nitrogen before the chamber opening is removed and the material introduced into the chamber. This provides for the necessary passage of samples in and out of the chamber without contaminating its nitrogen atmosphere with air or oxygen. The atmosphere of the extraction chamber was monitored frequently by gas-liquid chromatography over molecular sieve 5A and never exceeded an oxygen content of 0.1%.

e) Fractionation of freeze-dried meats. The glyceride fat and bound lipids were extracted from the freeze-dried meats as already described. In some cases, the bound lipids were further purified by washing or were treated with various additives. All operations were performed in a nitrogen atmosphere of the extraction chamber. Samples for oxygen absorption studies were placed into tar Warburg flasks in the chamber. The flasks were stoppered, removed from the chamber through the vacuum lock, weighed and immediately placed on the Warburg bath and flushed with oxygen.

2. Analytical methods

Measurements of moisture and of oxygen absorption were obtained as already described. Peroxide values were determined by iodometric procedure of Wheeler (1). Development of malonaldehyde w followed by a direct spectrophotometric method of Kwan and Watts (2) Fatty acid composition was obtained by gas-liquid chromatography on polyester column of the methyl esters of the fatty acids before and after hydrogenation. Odor changes were detected organoleptically b three observers. Samples were compared with controls and given a numerical rating from 0 (no odor change) to 5 (strong off-odors).

3. Moisture levels in freeze-dried meats

In order to study the effect of moisture content of d meat on its resistance to oxidation, it was necessary to prepare sa ples of the same meat containing different amounts of moisture. To provide a more uniform source of material, a single piece of rib eye or eye of the round was ground and thoroughly mixed before freeze drying. The ground meat was dried to a low moisture content and aliquots were allowed to partially rehydrate to higher moisture leve by storing them over various salt solutions in a vacuum desiccator i an atmosphere of nitrogen.

4. Rehydration of freeze-dried meat

In order to study the effect of storage in air on the ability of freeze-dried meat to reconstitute properly when treated with water, cubes of meat from a homogeneous sample were allowed to absorb water under standard conditions. A weighed cube of meat was immersed in water at 30°C. for exactly 15 minutes with the muscle fi in a vertical position. Excess moisture adhering to the surface of cube was removed by touching lightly each face of the cube with a pi of filter paper, and the cubes were weighed again to obtain the tota amount of water absorbed. Each cube was then introduced in a large test tube on a piece of screen rigidly supported about 3/4 of an inc above the bottom of the tube. The cubes were positioned with the fi parallel to the walls of the tubes and were centrifuged for 10 minut at 170 G. The cubes were again weighed and the loss in weight is co sidered a measure of the unbound or loosely bound water held by the meat.

C. Results

1. Composition of freeze-dried tissue

Table III shows the composition of three samples of freeze-dried tissue prepared in our laboratory from rib eye muscle from three different animals. The three samples differ markedly in their lipid content, but in all cases, the crude bound lipids constitute about 15% of the total lipids.

In Table IV the fatty acid composition of the glyceride fat and of the bound lipids extracted from beef rib eye sample No. 1 is shown. The major components of the glyceride fat were palmitic, palmitoleic, stearic and oleic acids, with smaller amounts of myristic, myristoleic, linoleic and linolenic acids. The bound lipids contained also major amounts of linoleic acid and dodecatrienoic acids. The components with carbon numbers 14.4, 15.4, 16.4 and 17.4 can represent either the monoenoic acid with 14, 15, 16 and 17 carbon atoms, respectively, or isoacid with one more carbon atom. In the case of the glycerides, these peaks disappeared upon hydrogenation and these components have been assumed to be the monounsaturated acids. The corresponding peaks arising from the fatty acids of bound lipids, however, were unchanged by hydrogenation, and in this fraction, these components have been labeled isoacids. No arachidonic acid was detected in either lipid fractions although attempts were made to find this expected fatty acid by greatly overloading the columns.

2. Absorption of oxygen

Figure 12 shows the absorption of oxygen by fractions obtained from freshly prepared freeze-dried meat from rib eye beef sample No. 1. As is the case with some of the commercial samples, the oxygen absorbed by the whole tissue during the initial oxidation period is almost completely accounted for by oxidation of the bound lipids. The tissue from which the glyceride fat has been removed by petroleum ether extraction but which still contains the bound lipids absorbs oxygen at the same rate as the isolated bound lipids during the entire course of the experiment. After approximately 500 hours, the whole tissue begins absorbing oxygen at a rate greater than can be accounted for by oxidation of the bound lipids alone. It would appear then that at this point the free glyceride portion of the fat must begin to oxidize, although this is much earlier than the time at which the isolated glyceride fat begins to oxidize, and at no time during the experiment does the over-all oxidation curve for the whole tissue resemble that of the free glyceride fat where a rapid oxygen absorption occurs after a long induction period.

Figure 13 shows similar data for rib eye sample No. 2. In both cases the isolated bound lipids absorb oxygen at identical rates, but the whole tissue from the second sample of rib eye beef absorbs oxygen much more rapidly than that of the corresponding tissue of the first sample, and at a rate much greater than can be accounted for by oxidation of the bound lipids only. The reasons for these differences is not known, but they may represent variation resulting from the fact that these tissues were obtained from two different animals.

3. Peroxides in oxidizing bound lipids

In earlier studies it was found that the fat extracted from freeze-dried meats that had absorbed appreciable amounts of oxygen contained no peroxides. Peroxide determinations were performed on lipids extracted from the oxidized tissue by petroleum ether. However, this solvent does not extract the bound lipids or their oxidation products, and since it was probable that only the bound lipids and not the triglyceride fat were oxidized when the peroxide determinations were performed, the possibility remained that peroxides were formed but were not extracted and, therefore, not measured. To verify this possibility, a sample of unoxidized bound lipids extracted from freshly prepared freeze-dried raw beef in an atmosphere of nitrogen was placed in a Warburg flask and allowed to oxidize until 162 m.e./kg. of oxygen had been absorbed. The sample was immediately subjected to the iodometric peroxide determination but no trace of iodine was liberated from the potassium iodide reagent. Therefore, although the bound lipids can absorb large amounts of oxygen rapidly, no peroxides accumulate during their oxidation and if peroxides are formed they must be very unstable and decompose immediately. The reasons for this are not clear. Although the stability of peroxides decreases with increasing unsaturation, peroxides accumulate in fish oil fatty acids with 5 or 6 double bonds oxidizing at temperatures in excess of 100°C. In contrast, the bound lipids were oxidized at 60° and contained no fatty acids with more than 3 double bonds. One possible explanation would be that these bound lipids contain highly efficient peroxide decomposers which prevent peroxide accumulation. Such compounds should be either prooxygenic if they catalyze peroxide decomposition with formation of new free radicals or antioxygenic if they destroy peroxide by reacting with them without formation of new free radicals. However, as will be shown later, water extracts of crude bound lipids are neither strongly prooxygenic nor antioxygenic, and if such peroxide decomposing substances are present in the crude bound lipids, they must remain with the lipids and are not extracted with water.

4. Effect of cooking on oxygen absorption

Figure 14 shows the oxygen absorption by freeze-dried raw and cooked whole tissue from the same sample of beef rib eye, and of the bound lipids extracted from these meats. In both cases, the cooked samples oxidize much faster than the raw material, the initial rates of oxygen absorption by the cooked samples being greater than those of the raw by a factor of about 2 for the whole tissue and of 3 for the extracted bound lipids.

The effect of cooking is shown also by Figure 15 where the glyceride fat extracted from the cooked meat has an induction period approximately 10% shorter than that of the corresponding fat obtained from the raw material. Cooking, therefore, increases the susceptibility of freeze-dried meat to oxidation and the effect is more severe on the bound lipids than on the glyceride fat. This is evident also by

comparing Figures 16 and 17 showing the rate of oxidation of bound lipids obtained from cooked and raw meat.

5. Development of malonaldehyde

It has been found that commercially prepared samples of freeze-dried meat did not show an increase in malonaldehyde content as a result of oxidation. However, since the history and level of oxidation of these samples were not known, it was felt that the formation of malonaldehyde as a result of autoxidation should be investigated in samples of freshly prepared freeze-dried meat which had not been previously exposed to oxygen. In a preliminary experiment, a sample of freshly prepared freeze-dried raw beef was found to contain 2.5 mg./kg. of malonaldehyde. After absorbing 10.7 m.e.O_2/kg. of fat the malonaldehyde content increased to 11.7 mg./kg. of sample.

Two additional experiments were performed in which samples of freeze-dried raw and cooked meat prepared in the laboratory were incubated in an oven at 40°C. and the malonaldehyde content was determined from time to time. The results in Table V show that there was no increase in malonaldehyde formation in the raw meat. In contrast, malonaldehyde accumulated rapidly in the cooked samples. This is in agreement with previous observations that cooking results in an accelerated rate of oxidation.

6. Development of off-odors in freeze-dried meat

The odors of the samples used for malonaldehyde determination were observed at regular intervals by three observers. For each observation, a fresh control of the same freeze-dried material was removed from storage at -20°C. in an atmosphere of nitrogen and allowed to warm up in the incubator at 40°C. for 5 minutes. Each sample was then compared directly with the control and the odor of the sample was rated on a scale from 0 (no off-odor) to 5 (strong off-odor) by each observer. The average numerical score for each sample is recorded in Table V also. Although no malonaldehyde developed in the raw freeze-dried beef, off-odors formed rapidly during incubation in air and continued to increase in intensity with length of incubation. Off-odors developed even more quickly and increased more rapidly in the cooked samples.

In another experiment bound lipids were extracted with chloroform: methanol in an atmosphere of nitrogen in the usual manner as described earlier. Each extract from the raw and cooked product was divided into four portions, each containing the extract from approximately 21 g. of freeze-dried meat in 150 ml. of solvent. The first portion was concentrated to 25 ml. without further treatment and 10 ml. aliquots were introduced into two tared Warburg flasks and the solvent removed in a stream of nitrogen and under vacuum. To fraction two was added 5 mg. of the disodium:calcium salt of ethylene diamine tetraacetic acid (EDTA-Na_2Ca) dissolved in 1/10 ml. of water. After thorough mixing,

this fraction was also concentrated to 25 ml. and 10 ml. aliquots introduced into Warburg flasks where they were freed of solvent. Fraction three was washed once by shaking it vigorously with 0.2 vol of distilled water, dried over anhydrous sodium sulfate and also reduced in volume to 25 ml. and aliquots transferred to Warburg flasks. The fourth fraction was washed in a similar manner but with a 2% aqueous solution of EDTA-Na_2Ca.

All eight Warburg flasks representing duplicates of each of the four fractions were placed in a Warburg bath at 60°C. One flask of each fraction was closed and oxygen absorption measurements were taken in the usual manner. The other flask was left open with a very slow stream of oxygen continously passing through it. Odor changes were detected by smelling the gases being washed out of the flasks by the oxygen stream at regular intervals by three observers. The over-all off-odors were rated on a scale from 0 to 5, as already described, but in addition, efforts were made to determine if any evidence of true rancidity could be detected in these gases. Results of this study are recorded in Tables VI and VII.

When all the samples of bound lipids were examined at room temperature immediately after they were removed from the nitrogen atmosphere of the special extraction chamber, they had a very mild, almost bland odor, similar to that of fresh meat. However, within a few minutes after they were placed in a 60° bath and exposed to oxygen they developed a definite, much stronger odor which was described as amine or acetamide odor, or as a meaty odor so strong that it was definitely unpleasant. The intensity of this odor increased with time and the character changed also, so that with all samples, it became more of a stale, sour, putrid type of odor. With the bound lipids from the raw meat, the odor from the unwashed material appeared to be slightly more intense than that of the washed product. However, the reverse was true with the extract from the cooked fraction.

In addition, the bound lipids which had been purified by washing with water or a solution of EDTA showed definite signs of rancid odor after 6 to 8 hours at 60°C. It might be suggested that the stronger odor shown by the cooked, washed bound lipids is due to the cumulative effect of the rancid odor component superimposed over the stale odor which first occurs in the freeze-dried meat. This explanation, however, cannot be advanced for the washed bound lipids from the raw meat since the rancid character of this washed fraction is even stronger than that of the fraction from the cooked meat while the over-all odor is weaker than that of the unwashed material.

7. Effect of washing and of sequestering agents on oxidation of bound lipids

Washing of the crude bound lipids with either water or an EDTA solution removed approximately 1/3 of this fraction as water-soluble non-lipid material. As shown in Tables VI and VII, the washed bound lipids oxidized more rapidly than the crude material and this effect is stronger for the bound lipids from the raw meat. From these

data it appears that the non-lipid water-soluble material removed from the crude bound lipids might be slightly antioxygenic. However, separate experiments demonstrated that the water-soluble fraction of the bound lipids had a strong amine type of odor, did not itself absorb oxygen, and was slightly prooxygenic when added to samples of glyceride fat extracted from freeze-dried meat.

The addition of EDTA directly to the crude bound lipids, also, has no great effect on the oxidation of this fraction. Apparently, if the rapid oxidation of the bound lipids is due to some metallic pro-oxidant, this is not in a form such that it can be complexed and inactivated under the conditions employed.

8. Effect of antioxidants on oxygen absorption

a) Effect on crude and washed bound lipids. Butylated hydroxyanisole (BHA) and DL-alpha-tocopherol were added in concentrations of 0.001 and 0.01%, respectively, to the crude and washed bound lipids extracted from raw and cooked freeze-dried beef. The oxygen absorbed by these fractions is shown in Figures 16 and 17. In all cases the data have been calculated in terms of purified washed bound lipids even though some of the experiments were performed on the crude material. Both tocopherol and BHA were effective in decreasing the rate of oxygen absorption of the crude and washed bound lipids obtained from raw freeze-dried beef. In no instance, however, was there evidence of an induction period. The samples containing antioxidants absorbed measurable amounts of oxygen from the beginning of the experiment at a constant initial rate that was, however, lower than that for the samples that were free of antioxidant. After approximately 80 mmoles O_2/kg. fat had been absorbed, the rate began to decrease until a second, much lower but also constant, rate had been obtained. Again the washed bound lipids oxidized more rapidly than the unwashed material and BHA was most effective in the unwashed samples. Both antioxidants had only a slight retarding effect on the washed bound lipids, and the substantial effect of BHA on the unwashed material may indicate the presence of a water-soluble synergist that was removed during the bound lipid washing procedure.

The effect of antioxidants on the bound lipids from the cooked meat are much smaller and not as clear-cut. Initially, the washed cooked bound lipids absorbed oxygen more rapidly than the unwashed control, but the rate of oxidation decreased more rapidly so that with time the unwashed material attained higher oxidation levels than the corresponding washed fractions. Instead of acting as antioxidants, tocopherol and BHA actually increased the rate of oxidations of the samples.

b) Effect on whole tissue. Various antioxidants, singly or in combination, were vacuum sprayed on raw and cooked freeze-dried meat and the oxygen absorption of the samples was determined. The use of these compounds did not change the shape of the oxygen absorption curves and none of the samples showed an induction period.

Although the initial rates of oxygen absorption were changed slight most of the added compounds resulted in increased rates of oxidatio The results obtained are shown in Table VIII where the time neces a for the samples to absorb 10 mmoles of O_2/kg. fat has been recorded

9. Effect of various polar substances on oxygen absorptio of whole tissue

The possibility exists that the rapid oxidative deteri oration of freeze-dried meat is due to molecular oxygen which is strongly adsorbed on active sites on which bound lipids are also adsorbed. It was thought that if freshly prepared freeze-dried me was first exposed to highly polar compounds, these might be adsorb on these active sites and block them from further attacks during sa sequent exposure to oxygen.

Small samples of ground beef were freeze-dried in test tube without exposure to oxygen, the vacuum was released with various p gases and vapors. The samples were then introduced into a Warburg apparatus and the absorption of oxygen was measured. The results o this study are shown in Figure 18. None of the polar compounds add protected the meat against autoxidation. Glycerol, carbon monoxide and carbon dioxide had no significant effect on the rate of oxidati while oxides of nitrogen, heptylamine and sulfur dioxide increased markedly the initial rate of oxygen absorption.

After 190 hours at 60°C., the odors of these samples were exam. by three observers. As shown in Table IX, the results indicate tha all treatments resulted in stronger off-odors in the samples. The sample with the least unpleasant odor was the control while treatmer with oxides of nitrogen gave a strongly rancid sample.

10. Effect of moisture on autoxidation

It is widely accepted that there exists an optimum mois ture level at which freeze-dried foods are most stable, and that dehydration to too low a moisture level results in increased instabi ity to oxidative deterioration and rancidity. It has been theorized that this optimum moisture content represents the amount of water necessary to form a monomolecular layer on active sites of the produ and thus protect it from attack by oxygen. However, it has been observed that the amount of water calculated to form a monomolecular layer on freeze-dried shrimp, chicken and beef represented a moistur level considerably higher than that recommended for best stability o these products (3). Several samples of freeze-dried beef containing different moisture contents were, therefore, prepared and their oxyg absorption measured at 60°C. Attempts were made to obtain different moisture levels by varying the length of freeze drying but, as alrea observed by other workers, this procedure was not very satisfactory. Instead, it was found more advantageous to freeze-dry the meat sampl to a very low moisture content and to partially rehydrate them by storing them over various saturated salt solutions.

In the first experiment, U. S. Good rib eye and eye of the round were obtained from the same animal. The meats were trimmed free of extraneous fat, chilled, ground once through a meat grinder, and thoroughly mixed by hand. Portions of 10 to 15 grams of the ground meat were packed at the bottom of a one-inch diameter test tube in a layer 3-5 ml. thick, frozen and freeze-dried at a pressure less than 1 mm. of mercury.

Samples of the ground rib eye were removed from the freeze drying manifold after 3, 4, 5, 6, 11 and 22 hours, at which times they contained respectively 13.7, 9.0, 8.1, 1.3, 0.51 and 0.07% moisture. The samples with the higher moisture contents did not appear uniform and seemed to contain some particles that were more wet than others. Therefore, all of the eye of the round samples were allowed to freeze dry for 26 hours, at which time the meat had a moisture content of 0.7%. The higher moisture level of the eye of the round samples after a longer dehydration period indicates that the ease with which water is removed is dependent on the type of tissue being dehydrated. The factors involved are probably not related directly to the type of muscle being used but perhaps to the fat content. Visual comparison of the two tissues indicated that the eye of the round contained more fat than the rib eye.

The freeze-dried eye of the round was partially rehydrated to moisture contents of 4.7 and 8.6% by storing the material in a nitrogen atmosphere over saturated solutions of calcium chloride for seven hours and sodium dichromate for twenty-four hours.

The results of the oxygen absorption measurements on these samples are shown in Figure 19. The results show that the four samples containing 1.3% moisture, or less, absorb oxygen at nearly identical rates. The sample containing 1.3% moisture oxidizes at a rate slightly lower than the other three low moisture samples, but the differences are very small and probably not significant. On the other hand, all the samples containing more than 4% moisture oxidized definitely more rapidly and generally the rate of oxidation increases with moisture content.

In a second experiment, U. S. Good rib eye and eye of the round were again obtained from the same animal, trimmed, ground and freeze-dried as already described. After ten hours the meat had moisture contents of 0.9% for the rib eye and 1.0% for round beef. These samples were partially rehydrated by storage over saturated solution of potassium acetate for seven hours, calcium chloride for eight hours, potassium acetate for forty-eight hours and saturated sodium dichromate for twenty-four hours to give the moisture contents shown in Figures 20 and 21. The oxygen absorbed by these samples again indicates that higher rates of oxidation accompany higher moisture content. In both types of tissue the rates of oxygen absorption when expressed in terms of total amount of sample are virtually identical for similar moisture levels.

Both meats contained an identical 6.8% of bound lipids and it ca be concluded that there is no significant difference between the two types of muscle as far as their oxidative deterioration is concerned.

The rates for the samples containing approximately 1% moisture are nearly the same as those shown in Figure 19 for the four samples containing less than 1.3% moisture. This similarity between these four samples obtained at different times from two animals and representing two types of muscle tissue suggests that moisture content may be a very important factor influencing the rate of oxidative deterioration of freeze-dried meat and that as low a moisture content as possi should be obtained.

11. Effect of type of muscle tissue on oxygen absorption

Rib eye muscle may be considered as mostly inactive tiss in which cellular metabolism would be expected to be low. In contras eye of the round, taken from the hind leg of the animal, may be regarded as a working muscle since it is almost constantly exercised every time the animal walks or moves and, therefore, metabolism in t tissue should be relatively high. The samples used in these studies were easy to distinguish visually since the eye of the round was of a deep dark red color while the rib eye meat was bright pink. It might be expected that the different rates of metabolism of these tissues and the amount or state of any heme pigments present might affect t e rates of oxidative deterioration of the freeze-dried tissues. Howev as shown in Figures 19, 20 and 21, there is no significant difference in the rates of oxygen absorption by samples of tissues at approximat equal moisture content.

12. Effect of storage in air on reconstitution of freeze-dri meat

Bound lipids are closely associated with the structural protein of tissue and have a strong influence on permeability of tiss membranes. It might be expected then that autoxidation of these boun lipids accompanied by formation of polar groups, polymers and lower molecular weight breakdown products might cause changes in the ease with which freeze-dried meats reabsorb water during reconstitution. It can be postulated that freeze-dried tissue would hold reconstitut water mainly in two different ways. First water more or less tight held by polar groups of the tissue through some type of physical-chem cal bond and, secondly, water mechanically held by capillary action within and between the bundles of muscle fibers. This latter type of water might be designated as loosely bound water which could be relatively easily removed from the tissue by squeezing or centrifugation.

To study the ease of reconstitution of tissues, cubes of freshly prepared freeze-dried meat were stored at room temperature and at 40° for various periods of time before they were reconstituted as describ under experimental procedures.

In the first experiment, cubes of raw and freshly cooked meat were centrifuged to determine the amount of loosely bound water in these undried samples, then they were freeze-dried and immediately rehydrated. Four cubes of raw meat and four cubes of cooked meat were used for each experiment and each cube was marked so it could be followed all through the process of centrifugation, freeze drying and reconstitution. The results of this experiment are shown in Table X.

The data indicate that cooking results in a loss of water and that both the raw meat and the freshly cooked meat contain appreciable amounts of loosely bound water which can be easily removed by centrifugation, the cooked meat containing approximately twice as much loosely bound water as the raw meat.

Under the conditions employed, the freeze-dried raw and cooked meats reabsorbed approximately two-thirds of their original water content. The reconstituted raw meat contained the same amount of loosely bound water as the original fresh meat and, therefore, the lower water content of the reconstituted meat represents a failure by the freeze-dried material to reabsorb nearly one-half of the more tightly held water of the tissues.

In the case of the cooked meat, however, the loosely held water of reconstitution was approximately half of the loosely held water present in the freshly cooked meat and the difference between the total water reimbibed and the original total water content of the cooked meat represents failure of the freeze-dried cooked product to reabsorb both bound water and loosely held water.

Freeze drying of the raw meat appears to have resulted in destruction of approximately one-half of the sites to which water may bind itself, but in no change in the capillary structure of the tissue. On the other hand, freeze drying of the cooked meat resulted in changes in both water binding sites and capillary structure.

In the second experiment, cubes of freshly freeze-dried raw and cooked beef were stored in air at 40°C. and at room temperature. At specific intervals, four cubes of each meat were removed and reconstituted using the standard procedure described.

The results of these experiments are in Table XI. There is some indication that storage of the raw freeze-dried beef, either at room temperature or at 40°C., results in a decrease in the amount of bound water absorbed by the reconstituted meat and in an increase in the amount of loosely held water. The data for freeze-dried cooked meat are more variable and in all cases the standard deviations are so high that the significance of the differences between the average values is doubtful.

For these reasons a third experiment was performed for which 8 cubes of meat were used for each measurement and the storage times were extended to 1 week, 2 weeks and 4 weeks at 40°C. The results

recorded in Table XII show again that storage resulted in a decrease in the bound water reabsorbed by raw and cooked freeze-dried meat and in an increase in the amount of loosely held water in reconstituted raw meat. Storage, however, had little effect on the amount of loose held water in reconstituted cooked meat.

In all cases standard deviations are again quite high, reflecting a great variability in the rehydration ability of individual pieces of meat. In each experiment the individual cubes used for the measurements were selected at random from the same piece of muscle. In view of the very similar oxidation characteristic of freeze-dried meat obtained from rib eye or eye of the round from the same animal, it would be expected that samples obtained from a single muscle would also show nearly identical autoxidation behavior. The differences shown in individual pieces during reconstitution, therefore, may indicate that either autoxidation of the bound lipids has little effect on reconstitution behavior or that the method used for this study leaves much to be desired from the point of view of reproducibility and reliability.

IV. CONCLUSIONS

1. Freeze-dried meats when exposed to air undergo rapid autoxidation and quickly develop stale off-odors.

2. The initial rapid oxygen absorption of these products is due to autoxidation of the bound lipids whereas the free glyceride fats are quite resistant to autoxidation and do not appear to be a factor in the development of stale off-odors.

3. Although the bound lipids oxidize rapidly, no peroxides accumulate. Malonaldehyde has been found to develop during oxidation of cooked meat but not of raw meat.

4. Antioxidants do not prevent oxygen absorption by bound lipids or by whole freeze-dried meat and show only a small effect in retarding the rate at which oxygen is absorbed by these materials.

5. Washed bound lipids develop typical rancid odors while the crude bound lipid fractions have an amine-type, putrid-like odor, which is derived mostly from a water-soluble non-lipid material.

6. The washed and crude bound lipids absorb oxygen at approximately the same rate. In the crude fraction the rancid odor of the washed bound lipids may be either masked by the odor of the non-lipid material, or the course of oxidation is changed so that rancidity does not develop, or the aldehydes responsible for rancid odors react immediately with other components of the crude bound lipids.

7. Attempts to prevent autoxidation of freeze-dried meats by treating them with various polar compounds, to bind and remove from attack by oxygen active autoxidation sites, were unsuccessful.

8. A non-working and a working muscle from the same animal autoxidized at the same rate, and showed no difference in this respect.

9. Moisture content of freeze-dried meat appears to be a very important factor influencing the rate of oxidation of these products.

10. Storage of freeze-dried meats in air results in decreased rehydration ability during reconstitution with water.

11. For best stability and palatibility it is suggested that:

a) meat should be freeze-dried to a moisture level of 1% or less;

b) the freeze-dried product should not, at any time, be exposed to oxygen, and should be kept under vacuum or in an inert atmosphere;

c) the product be stored at low temperature; and

d) that it be used as quickly as possible.

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Table I

Description of Commercially Prepared Freeze-Dried Samples

Item	Date of Pack	Packer	Lot
Beef, Ground, Precooked, Dehy., type I	8 Nov 60	Armour	210
Beef, Ground, Precooked, Dehy., type I	28 Oct 60	Armour	25
Fish Squares, Raw, Dehy., Style 2, Species B	24 Feb 61	Wilson	13
Fish Squares, Raw, Dehy., Style 2, Species B	10 Feb 61	Wilson	5
Beef (For Stew), Raw, Dehy.	28 Apr 61	Armour	B10117
Beef (For Stew), Raw, Dehy.	28 Apr 61	Armour	B12118
Beef Steaks, Raw, Dehy., type IV	16 Sep 60	Wilson	2
Beef Steaks, Raw, Dehy., type IV	27 Sep 60	Wilson	3
Beef Steaks, Raw, Dehy., type IV	9 Jun 60	Wilson	3
Beef Flaked Steaks, Raw, Dehy.	19 Sep 61	Wilson	1
Beef Steak, Raw, Dehy., type I	3 Mar 61	Wilson	8
Beef Steak, Raw, Dehy., type I	1 Feb 60	Wilson	3
Boneless Beef Steak from Beef Rib Eye, Raw	Oct 56	Armour	R11410
Beef, Cooked, Sliced, type I	27 Dec 61	Liana	36114
Beef, Cooked, Sliced, type I	Feb 60	Wilson	1
Beef, Cooked, Dehy., type II	Mar 60	Wilson	2
Beef, Cooked, Dehy., type II	13 Apr 61	Armour	E1102
Beef, Cooked, Dehy., type II	18 Apr 61	Armour	E2107
Beef, Cooked, Dehy., type III	26 Jun 58	Wilson	5
Beef, Cooked, Dehy., type III	24 Oct 61	Liana	29811
Beef, Cooked, Dehy., type III	24 Oct 61	Liana	29814
Beef, Cooked, Dehy., type III	31 Oct 61	Liana	30417
~~Beef Pot Roast, Cooked~~	~~Oct 61~~	~~Liana~~	~~29711~~
Beef Pot Roast, Cooked	Oct 61	Liana	29716
Pork Chops, Raw, Dehydrated	24 Mar 61	Armour	A11 82
Pork Chops, Raw, Dehydrated	23 Mar 61	Armour	A8 81
Fish Sticks, Style 1, Species B, Raw	30 Apr 60	Wilson	1
Shrimp, Raw, Dehydrated	Nov 61	Liana	30612

Table II

Composition of Freeze-Dried Meats

Sample	Moisture	Glyceride Fat	Bound Lipids	Total Lipids
Cooked Products				
Ground Beef, Type I Armour Lot 210	0.3	46.7	4.0	50.7
Ground Beef, Type I Armour Lot 25	0.4	39.5	6.2	45.7
Sliced Beef, Type I Liana Lot 36114	0.9	27.0	4.3	31.3
Sliced Beef, Type I Wilson Lot 1, 2/60	1.7	25.8	5.2	31.0
Beef, Type II Wilson Lot 2, 3/60	1.1	17.1	6.0	23.1
Beef, Type II Armour Lot E1102	0.8	30.0	4.8	34.8
Beef, Type II Armour Lot E2107	0.5	43.1	3.4	46.5
Beef, Type III Wilson Lot 5, 6/26/58	0.5	33.2	3.6	36.8
Beef, Type III Liana Lot 29811	1.3	39.2	3.2	42.4
Beef, Type III Liana Lot 29814	1.2	30.3	4.4	34.7
Beef, Type III Liana Lot 30417	1.8	29.4	4.3	33.7
Beef Pot Roast Liana Lot 29711	1.1	25.0	4.3	29.3
Beef Pot Roast Liana 29716	1.2	30.3	4.8	35.1
Raw Products				
Beef (for stew) Armour Lot B10117	1.0	22.7	6.4	29.1
Beef (for stew) Armour Lot B12118	1.3	36.5	5.9	42.4

Table II continued
Composition of Freeze-Dried Meats

Sample	Moisture	Glyceride Fat	Bound Lipids	Total Lipids
Raw Products				
Beef Steaks, Type IV Wilson Lot 2, 9/16/60	2.5	21.8	8.9	30.7
Beef Steaks, Type IV Wilson Lot 3, 9/27/60	1.0	14.4	8.7	23.1
Beef Steaks, Type IV Wilson Lot 3, 6/9/60	1.0	13.1	7.8	20.9
Beef Flaked Steaks Wilson Lot 1, 9/19/61	0.7	38.9	8.1	47.0
Beef Steaks, Type I Wilson Lot 8, 3/3/61	1.0	36.3	6.7	43.0
Beef Steaks, Type I Wilson Lot 3, 2/1/60	2.1	35.4	6.6	42.0
Boneless Beef Steak (Rib Eye) Armour Lot R11410	0.8	34.9	5.4	40.3
Pork Chops Armour Lot All 82	0.4	42.2	5.5	47.7
Pork Chops Armour Lot A8 81	0.7	44.8	4.9	49.7
Fish Squares, Style 2 (Species B) Wilson Lot 13, 2/24/61	3.0	0.6	8.7	9.3
Fish Squares, Style 2 (Species B) Wilson Lot 5, 2/10/61	3.1	0.9	12.1	13.0
Fish Sticks, Style 1 (Species B) Wilson Lot 1, 4/30/60	0.9	0.9	10.8	11.7
Shrimp Liana Lot 30612	1.6	2.4	12.3	14.7

Table III

Composition of Experimental Rib Eye Beef

Analysis	Sample 1	Sample 2		Sample 3	
	Raw	Raw	Cooked	Raw	Cooked
Weight loss on cooking	-	-	23.2(1)	-	12.4(1)
Weight loss on drying	-	72.3(1)	65.8(2)	68.1(1)	63.9(2)
Moisture in dried material(3)	3.3	0.8	0.8	1.3	0.7
Glyceride fat(3)	34.7	13.2	14.9	24.5	32.6
Crude bound lipids(3)	6.0	3.5	3.2	4.4	4.4
Washed bound lipids(3)	-	2.3	2.4	3.0	3.0

(1) % of raw fresh weight

(2) % of freshly cooked weight

(3) % of dried weight

Table IV

Fatty Acid Composition of Glyceride Fat and Bound Lipids of Freeze-Dried Beef

Peak Carbon Number	Fatty Acid	Amount (%)	
		Glyceride	Bound Lipids
10.0	10:0	tr.	--
12.0	12:0	tr.	--
13.3	13:1	--	1.2
14.0	14:0	3.0	1.1
14.4	14:1	1.0	--
14.4	iso 15:0 (?)	--	0.3
15.0	15:0	tr.	0.4
15.4	iso 16:0 (?)	--	6.5
16.0	16:0	30.8	18.4
16.4	16:1	6.3	4.2
17.0	17:0	0.8	0.4
17.4	17:1	0.8	--
17.4	iso 18:0 (?)	--	3.1
18.0	18:0	10.5	10.0
18.4	18:1	44.3	31.7
19.0	18:2	1.1	12.6
19.8	18:3	0.9	0.7
21.5	unknown	--	2.3
21.9	20:3	--	7.0

Table V

Development of "Off-Odors" and of Malonaldehyde in Freeze-Dried Beef

Time at 40°C.	Raw		Cooked	
	Average Odor Score*	Malonaldehyde Content	Odor Score	Malonaldehyde Content
hrs.		mg/kg.		mg/kg.
0	0	7.0	0	8.9
24	1.7	-	2.0	-
48	1.0	9.1	2.0	29.7
72	2.3	-	3.0	-
96	2.7	8.9	3.3	31.7
0	0	8.3	0	16.2
9	1.7	9.5	2.7	24.7
14	1.7	8.9	2.7	33.3
18	2.1	-	3.0	43.3

*0 = no "off-odor"; 5 = strong "off-odor"

Table VI

Odor and Oxygen Absorption of Bound Lipids of Freeze-Dried Raw Beef

Sample	Time at 60°C. hrs.	Oxygen Absorbed m.e./kg.	Average Odor Score*	Organoleptic Rancidity
Crude bound lipids	0	0	1.0	0
	2	8.1	2.3	0
	4	17.5	3.0	0
	6	25.2	3.7	0
	8	30.5	3.7	0
Crude bound lipids + EDTA	0	0	1.0	0
	2	10.9	2.3	0
	4	20.7	3.3	0
	6	29.5	4.3	0
	8	37.0	3.7	0
Water washed bound lipids	0	0	1.3	+
	2	24.7	2.0	++
	4	43.1	2.0	++
	6	53.1	2.3	++
	8	62.1	3.0	++
EDTA washed bound lipids	0	0	1.3	+
	2	38.3	2.0	++
	4	54.3	2.0	++
	6	66.7	2.3	++
	8	74.2	3.0	++

*0 = no "off-odor"; 5 = strong "off-odor"

Table VII

Odor and Oxygen Absorption of Bound Lipids of Freeze-Dried Cooked Beef

Sample	Time at 60°C. hrs.	Oxygen Absorbed m.e./kg.	Average Odor Score*	Organoleptic Rancidity
Crude bound lipids	0	0	0.7	0
	2	11.7	1.3	0
	4	20.4	2.0	0
	6	29.6	2.0	0
	8	38.4	2.7	0
Crude bound lipids + EDTA	0	0	1.0	0
	2	15.6	1.7	0
	4	30.8	1.3	0
	6	41.7	1.3	0
	8	50.3	2.0	0
Water washed bound lipids	0	0	1.3	0
	2	25.2	2.7	0
	4	37.3	3.3	+
	6	44.4	3.0	+
	8	52.1	4.0	++
EDTA washed bound lipids	0	0	1.3	0
	2	16.8	2.3	0
	4	28.8	2.7	+
	6	39.4	3.3	+
	8	43.8	4.3	++

*0 = no "off-odor"; 5 = strong "off-odor"

Table VIII

Effect of Antioxidant on Autoxidation of Freeze-Dried Meats

Antioxidant Added	Time to Absorb 10 mmole O_2/kg. of Fat	
	Raw (hours)	Cooked (hours)
None (control)	95	120
α-Tocopherol	71	100.5
Propyl gallate (PG)	105	119
Butylated hydroxyanisole (BHA)	110	126
Citric acid (CA)	102.5	109
Sodium tripolyphosphate ($Na_5P_3O_{10}$)	82.5	114
BHA, PG, CA	84	84
BHA, PG, $Na_5P_3O_{10}$	62.5	37.5

Table IX

Effect of Various Polar Substances on Development of Odors in Freeze-Dried Raw Beef

(odors after 190 hours at 60°C. in an atmosphere of oxygen)

Sample and treatment	Odor
Control	Slightly putrid, least of all samples
Carbon monoxide	Slightly putrid
Carbon dioxide	Slightly sour, less putrid
Glycerol	Somewhat sweet odor
Sulfur dioxide	Sharp odor
Heptylamine	Strong amine odor
Oxides of nitrogen	Strongly rancid

Table X

Water in Fresh Meat and Reconstituted Freeze-Dried Meat

Water and Meat	Raw	Cooked
Fresh Meats		
Loosely held water (% of dry matter)	28.5 (8.5)*	54.4 (9.5)
Bound water (% of dry matter)	206.8 (18.6)	155.3 (11.4)
Reconstituted Freeze-Dried Meats		
Loosely held water (% of dry matter)	28.6 (8.8)	24.4 (8.2)
Bound water (% of dry matter)	118.1 (16.9)	116.4 (13.2)

*Average of 4 determinations and standard deviation (in parentheses)

Table XI

Effect of Storage in Air at Room Temperature and 40°C. on Rehydration of Freeze-Dried Beef

Storage Conditions	Bound water (% of dry matter)		Loosely held water (% of dry matter)	
	Raw	Cooked	Raw	Cooked
Room Temperature				
0 week	114.3 (24.7)*	97.6 (15.2)	39.6 (11.5)	34.1 (11.9)
1 week	114.8 (19.7)	112.2 (8.7)	45.3 (11.7)	52.2 (6.2)
2 weeks	115.4 (20.0)	100.9 (16.2)	50.0 (3.5)	38.1 (6.3)
4 weeks	108.0 (10.3)	92.6 (9.4)	47.5 (13.8)	40.9 (3.2)
40°C.				
0 day	114.3 (24.7)	97.6 (15.2)	39.6 (11.5)	34.1 (11.9)
1 day	113.0 (19.1)	105.6 (10.8)	29.2 (14.1)	35.4 (6.5)
2 days	104.8 (8.2)	126.2 (46.5)	46.4 (6.9)	47.3 (9.8)
4 days	93.3 (30.8)	109.5 (15.1)	38.1 (16.6)	46.0 (12.1)

*Average of 4 determinations and standard deviation (in parentheses)

Table XII

Effect of Storage in Air at 40°C.

on Rehydration of Freeze-Dried Raw and Cooked Beef

Storage Time (Weeks at 40°C.)	Bound Water (% of dry matter)		Loosely held water (% of dry matter)	
	Raw	Cooked	Raw	Cooked
0	99.1 (14.6)*	107.3 (7.5)	35.4 (9.7)	38.2 (3.7)
1	103.1 (20.6)	98.9 (9.4)	47.6 (7.9)	42.0 (8.0)
2	91.7 (10.8)	77.9 (18.1)	40.6 (8.1)	36.2 (8.7)
4	88.3 (25.4)	70.9 (18.0)	43.9 (21.8)	39.3 (16.2)

*Average of 8 determinations and standard deviation (in parentheses)

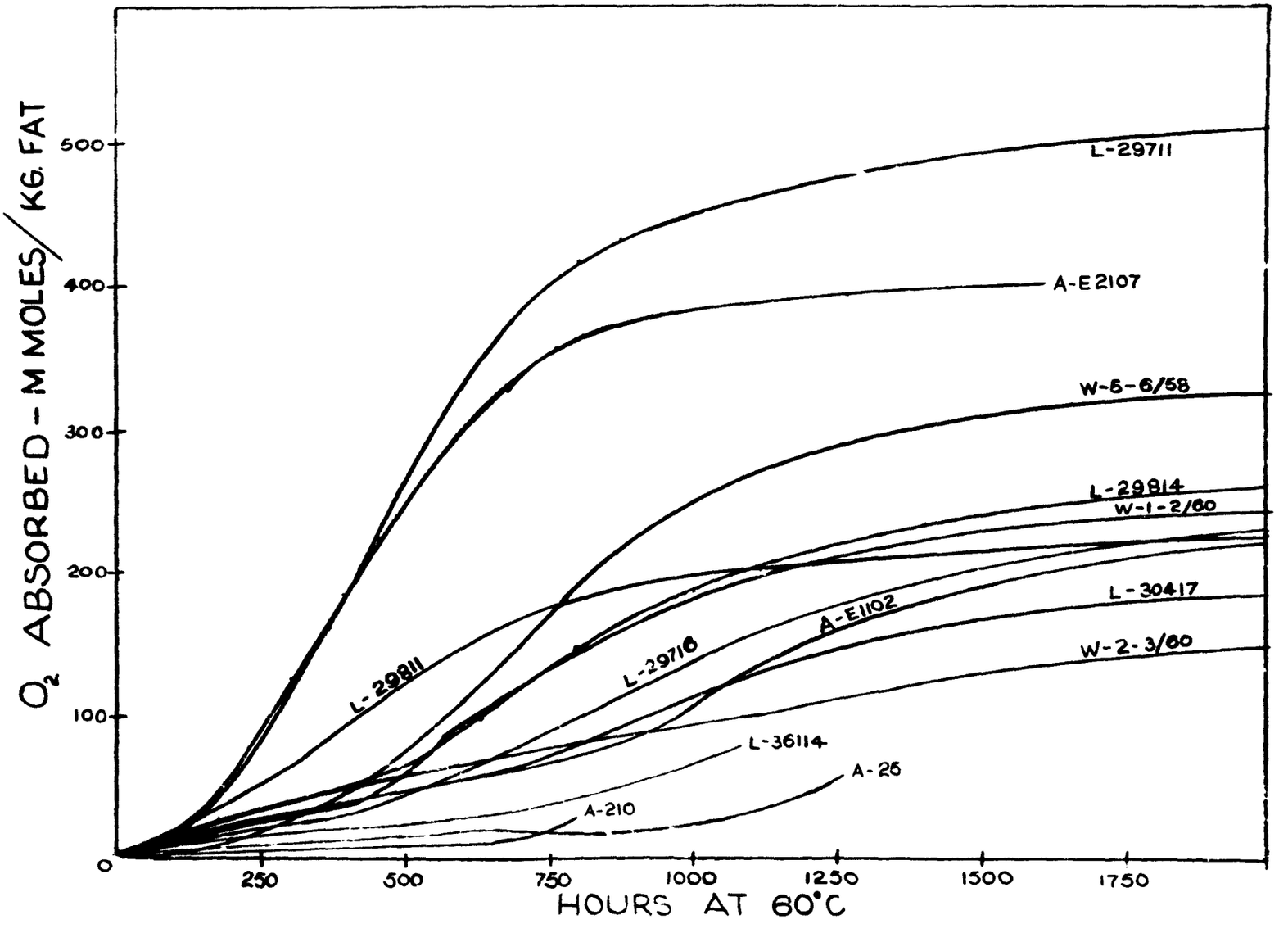

FIG. 1 - OVERALL OXYGEN ABSORPTION BY COOKED WHOLE TISSUE

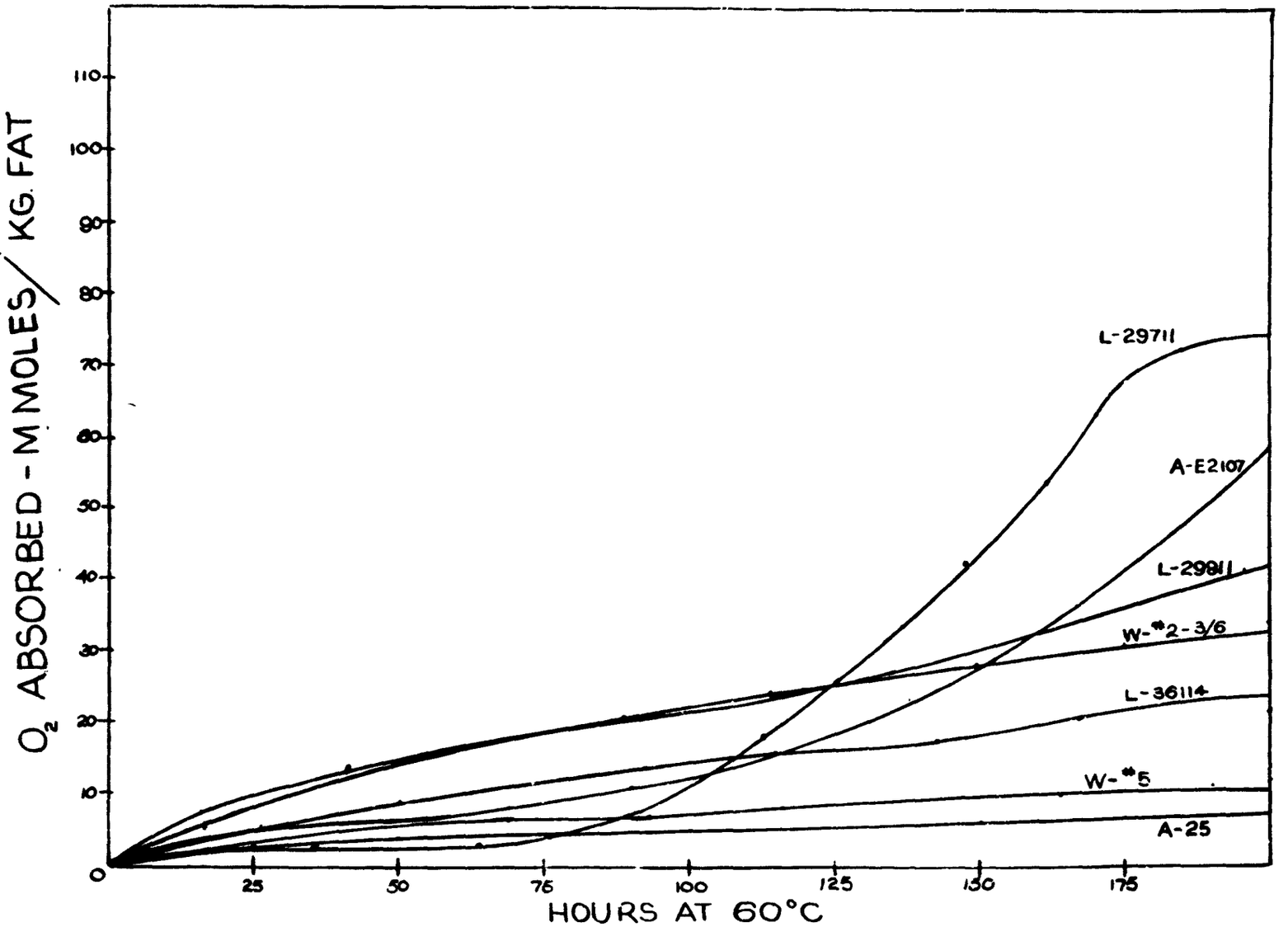

FIG. 2-INITIAL OXYGEN ABSORPTION BY COOKED WHOLE TISSUE

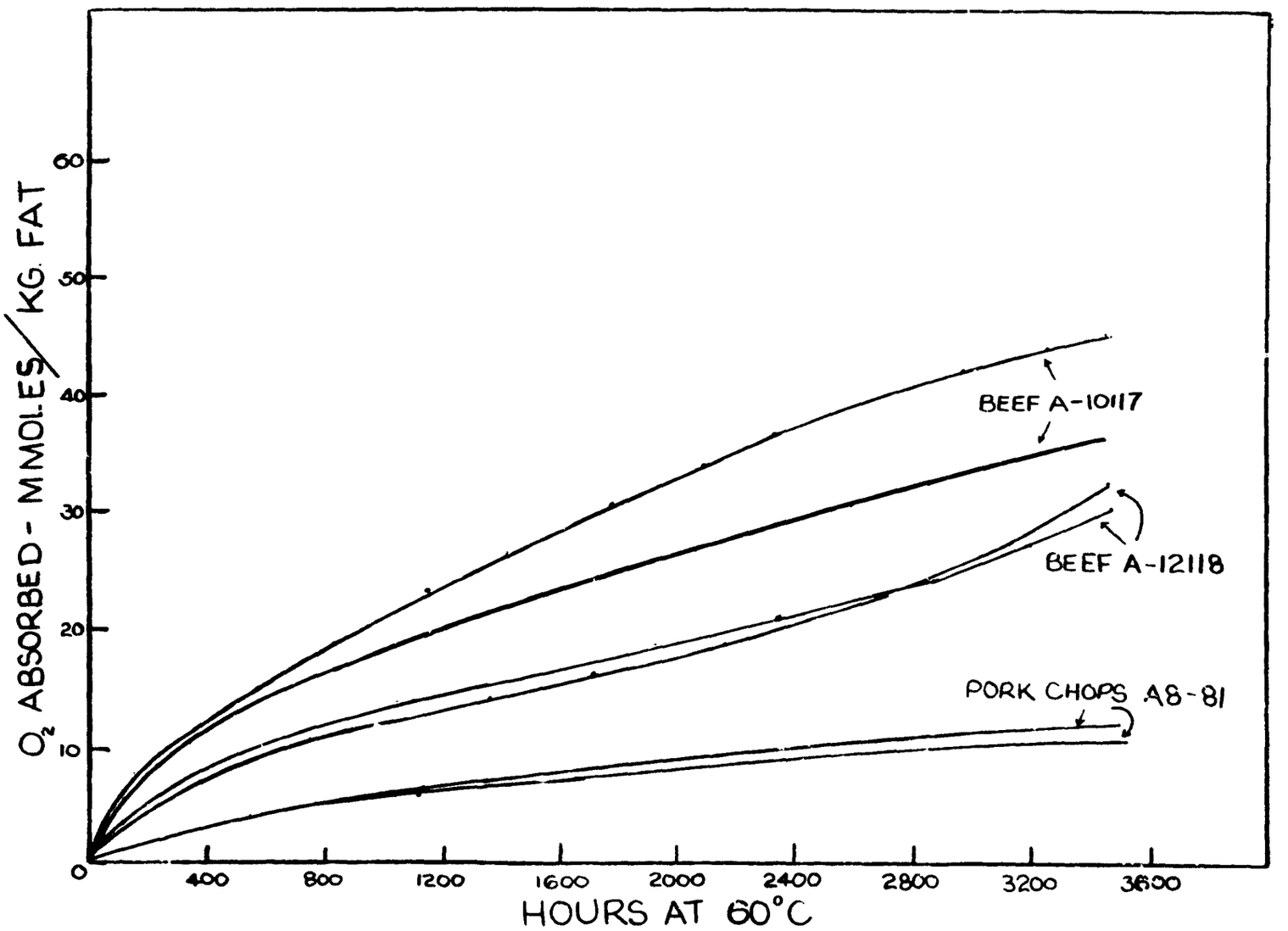

FIG 3 - OXYGEN ABSORPTION BY DUPLICATE PORTIONS OF HOMOGENEOUS SAMPLES

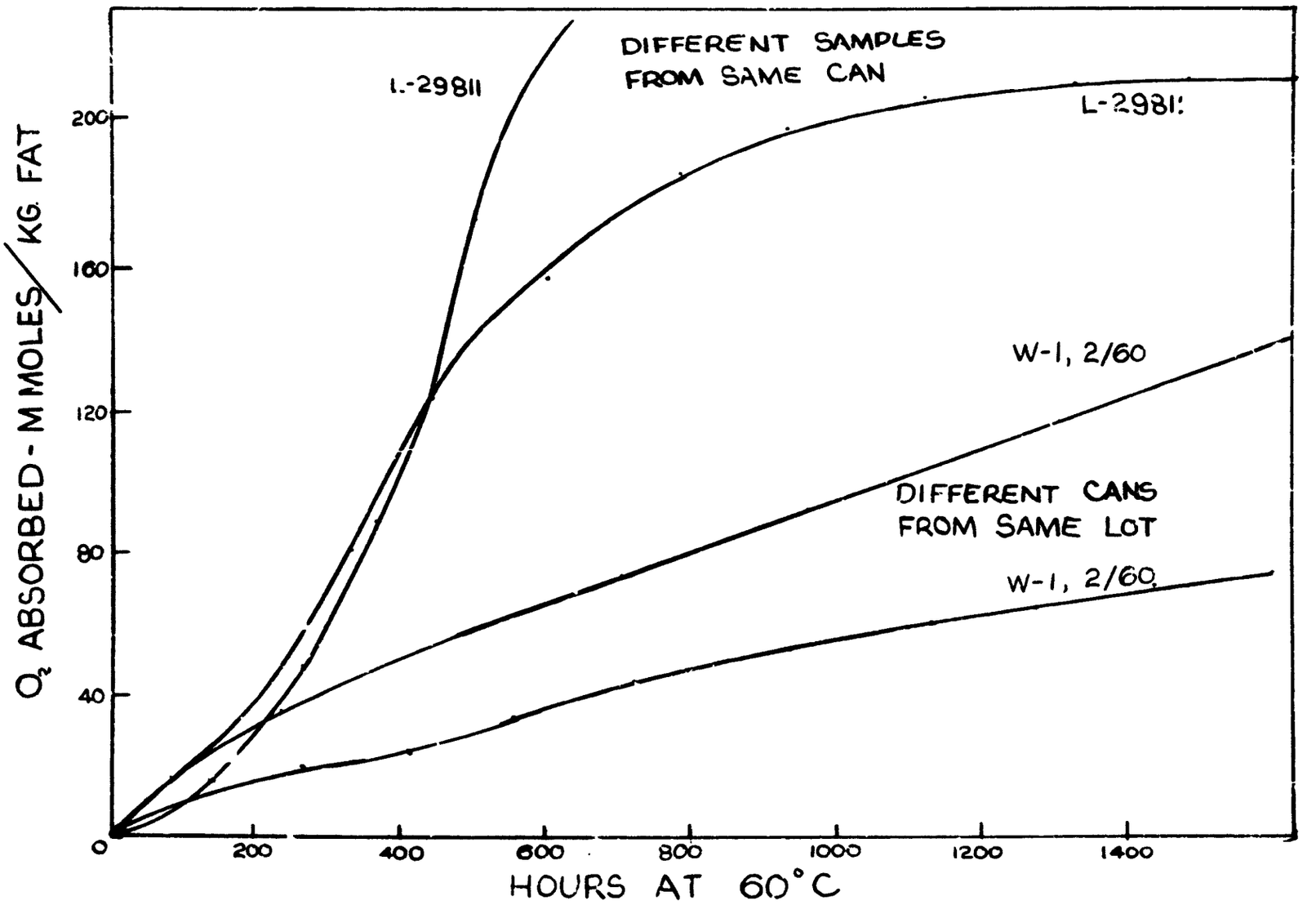

FIG 4 OXYGEN ABSORPTION BY DIFFERENT SAMPLES FROM SAME LOT

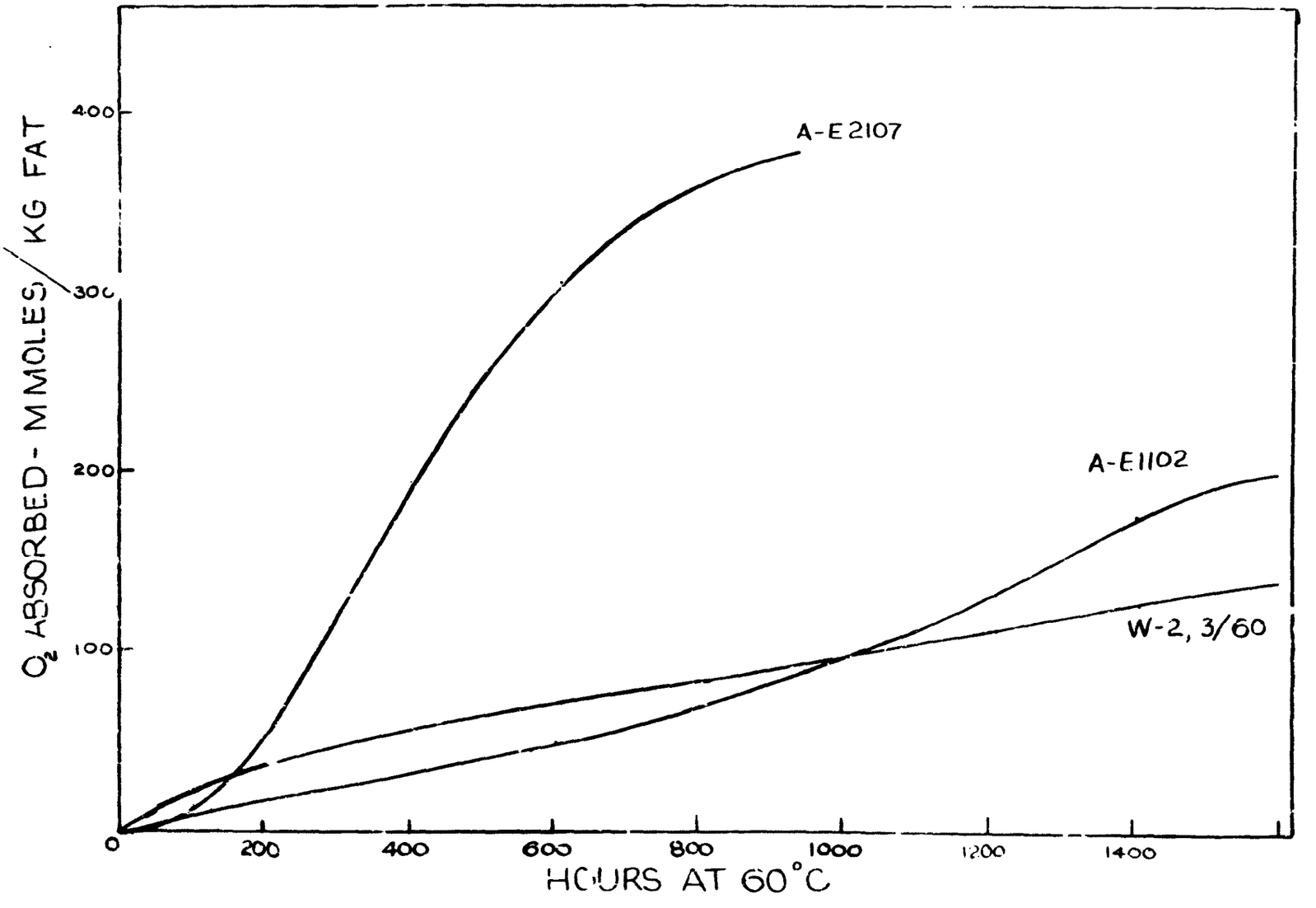

FIG. 5 - OXYGEN ABSORPTION BY VARIOUS LOTS OF SAME TYPE OF PRODUCT

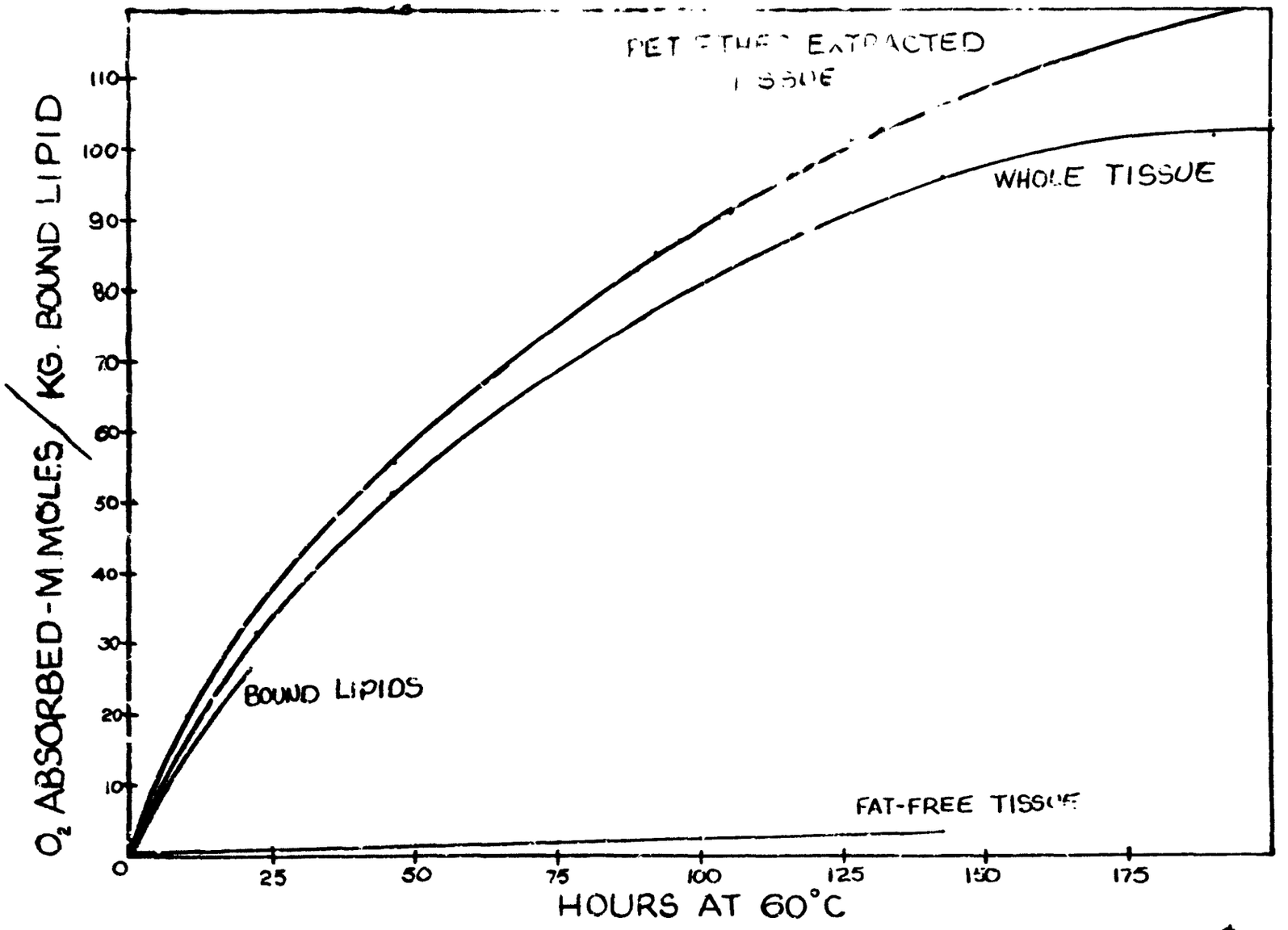

FIG. 6- ABSORPTION OF OXYGEN BY FRACTION FROM W-*1-2/60

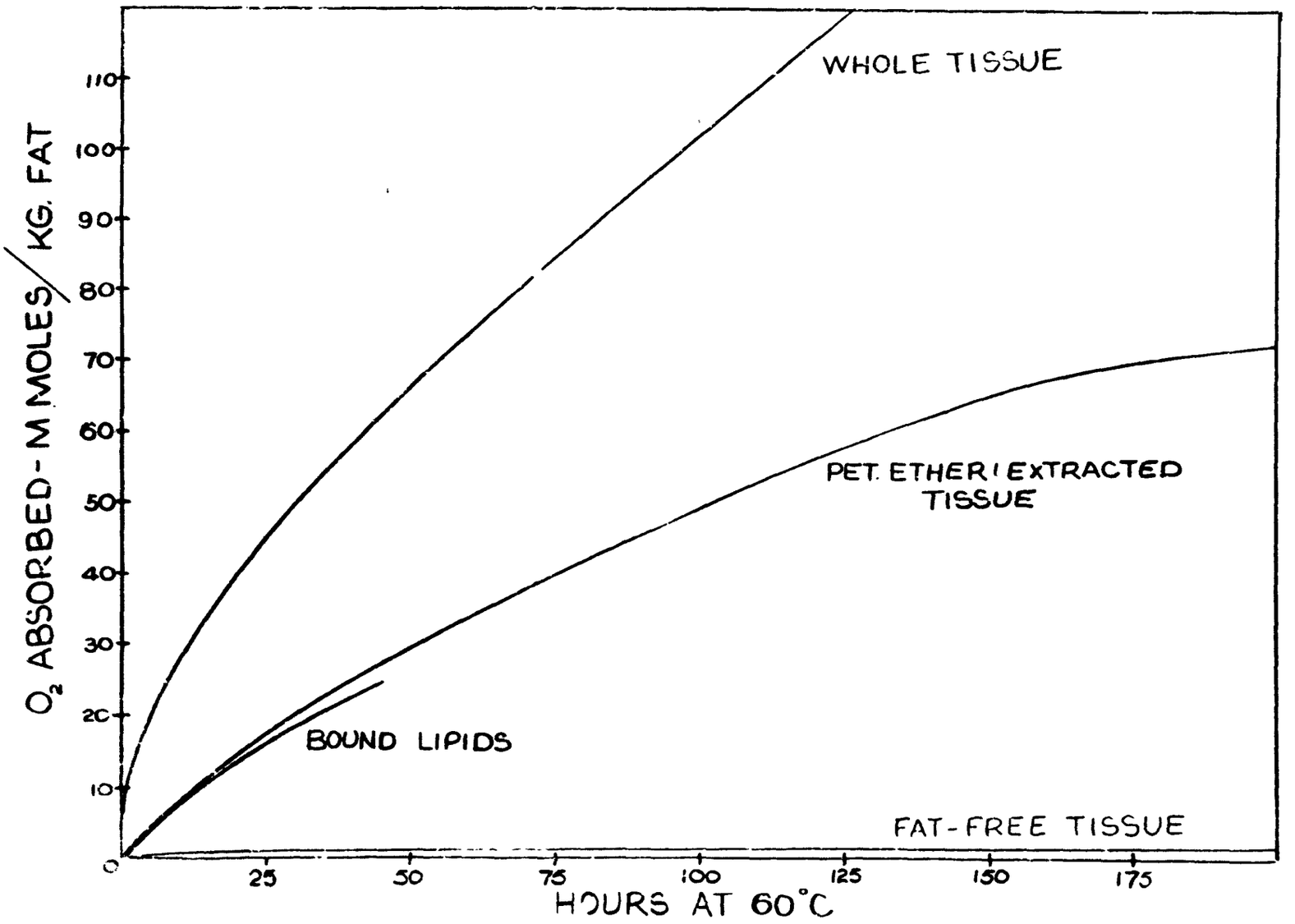

FIG. 7 - ABSORPTION OF OXYGEN BY FRACTIONS FROM L-30114

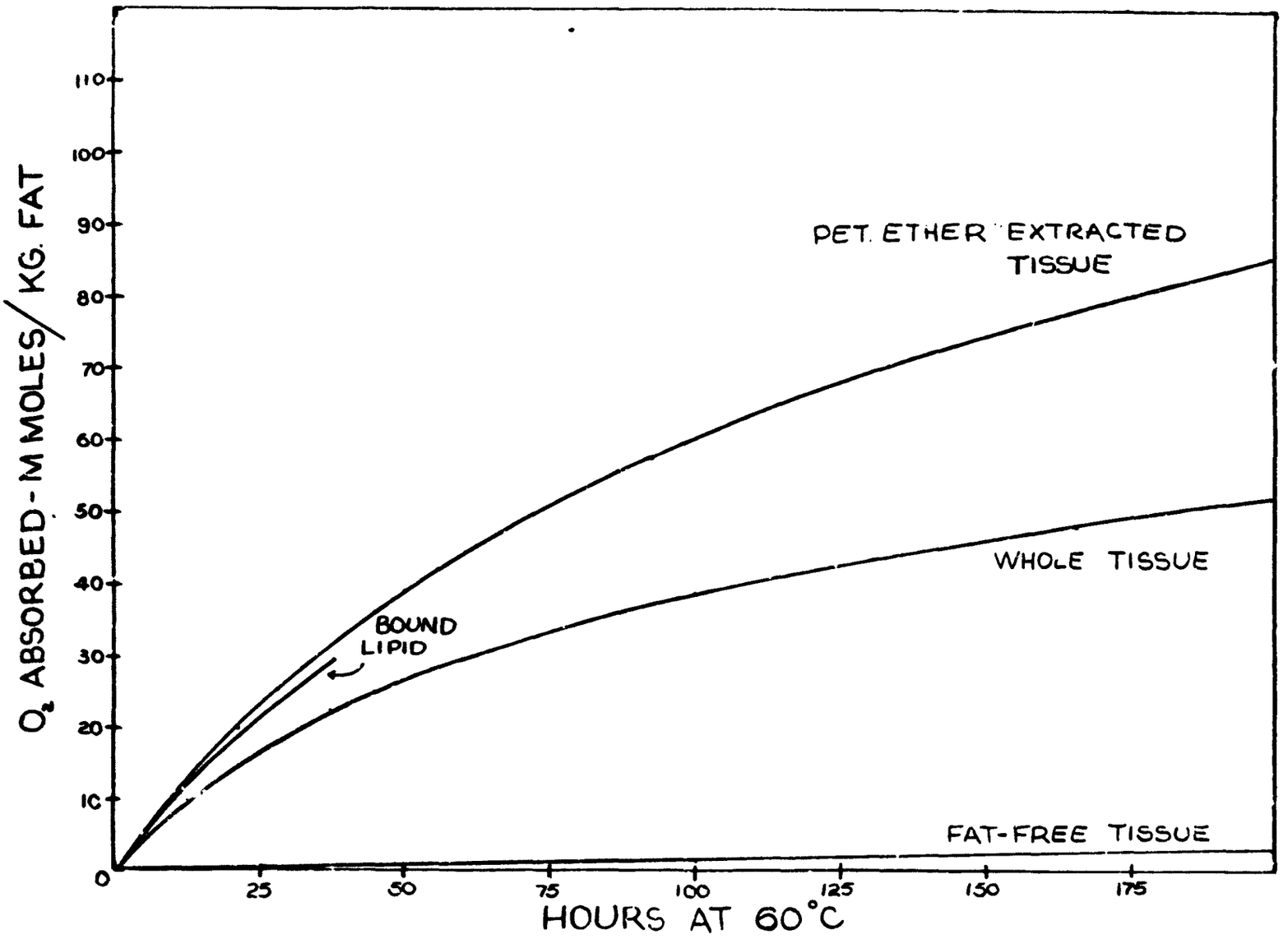

FIG. 8- ABSORPTION OF OXYGEN BY FRACTIONS FROM A-25

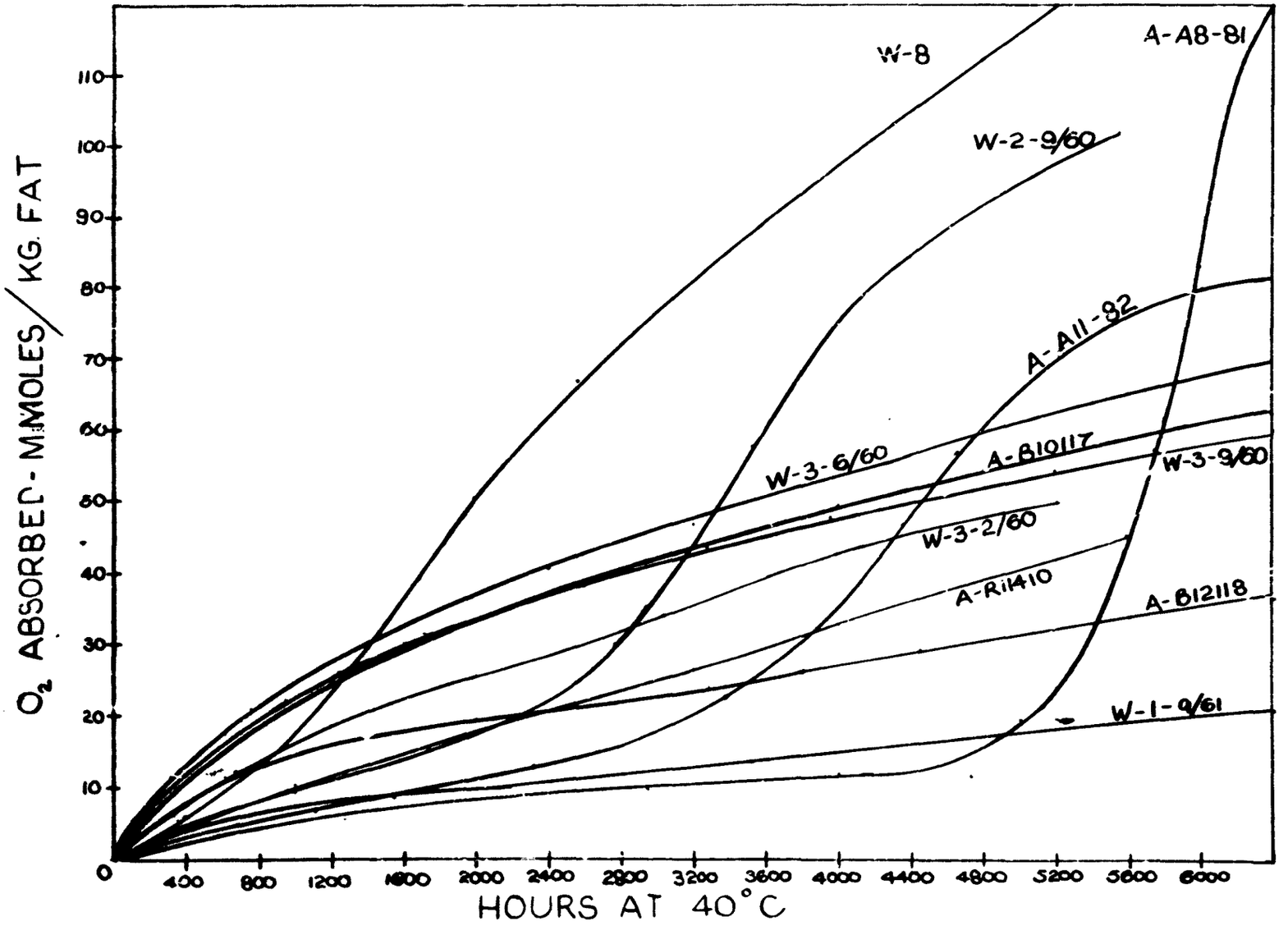

FIG. 9 - OVERALL OXYGEN ABSORPTION BY RAW WHOLE MEATS

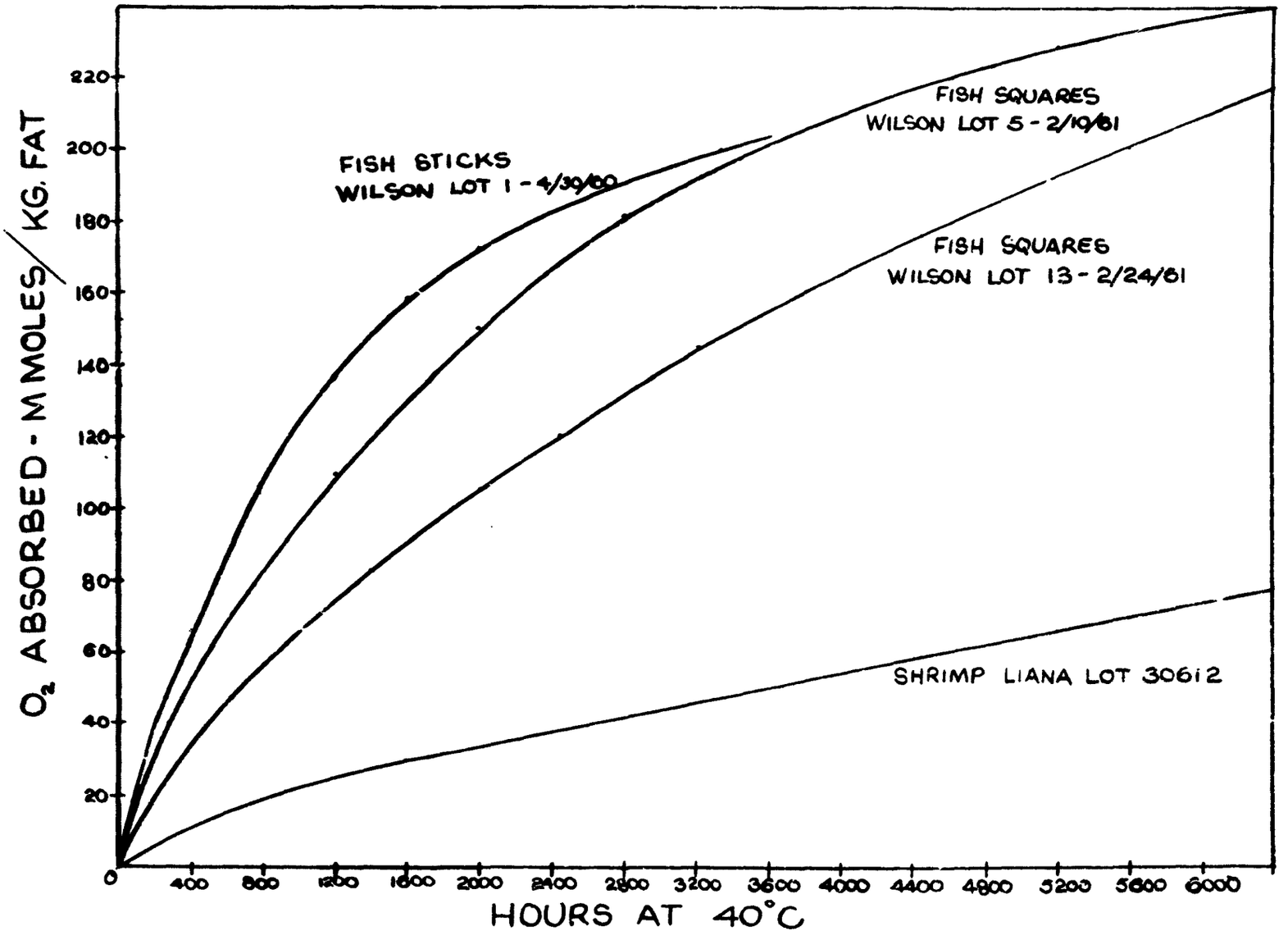

FIG. 10- ABSORPTION OF OXYGEN BY FREEZE-DRIED FISH

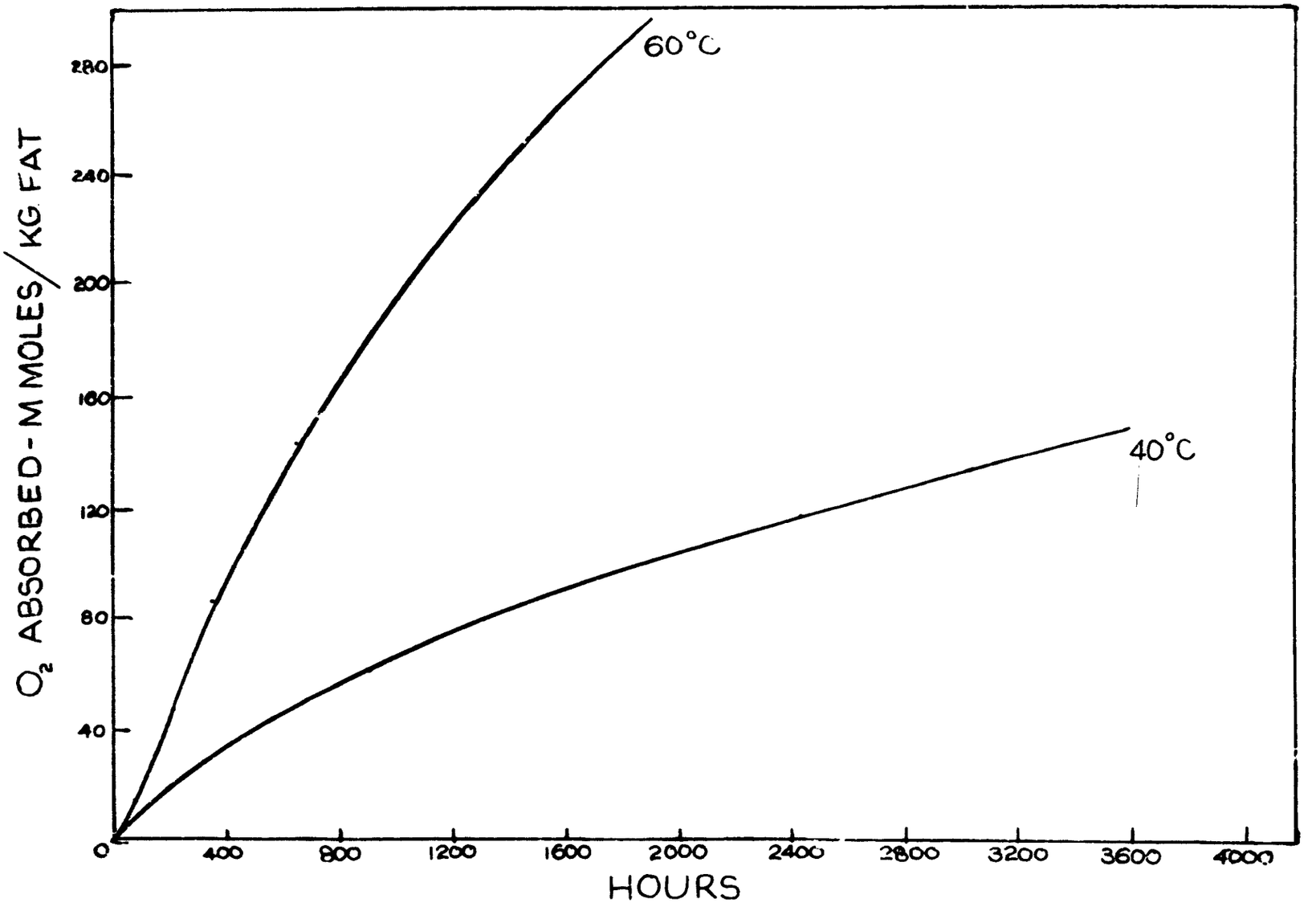

FIG. 11 - OXIDATION OF RAW FISH SQUARES (WILSON LOT 13) AT 40° AND 60°C.

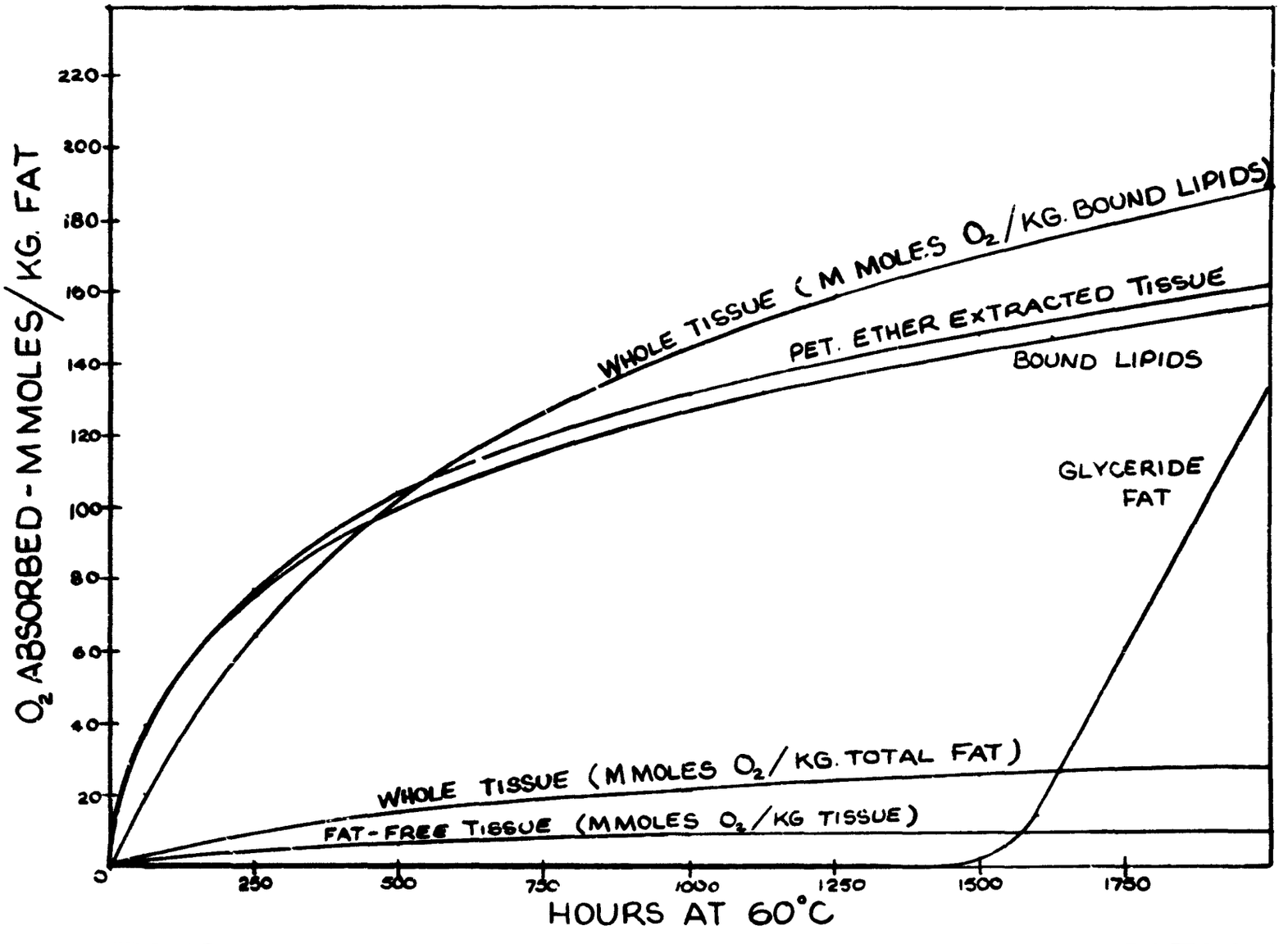

FIG. 12 - ABSORPTION OF OXYGEN BY RIB EYE LABORATORY

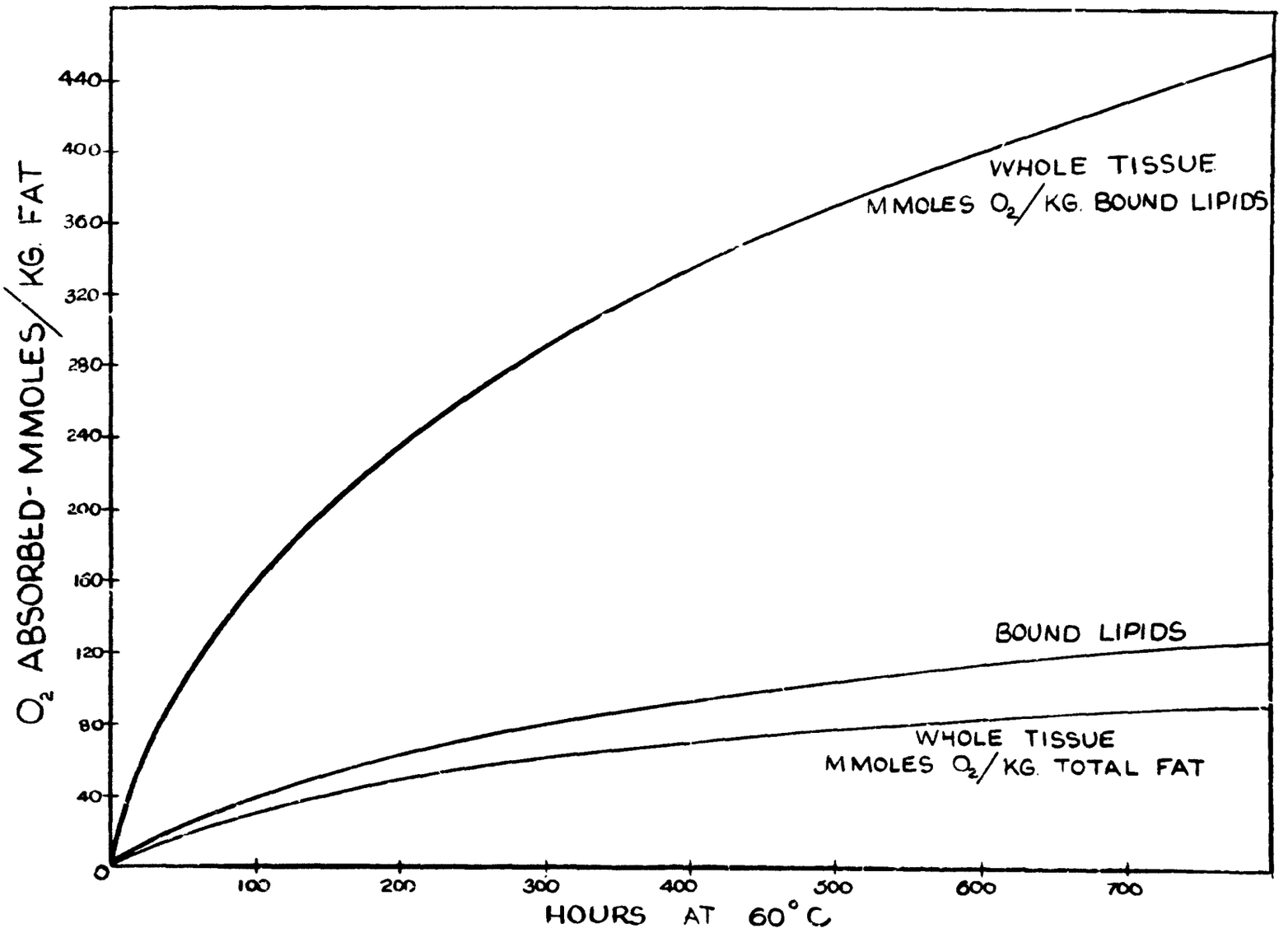

FIG. 13 - OXYGEN ABSORBED BY RAW RIB EYE LABORATORY SAMPLE NO. 2

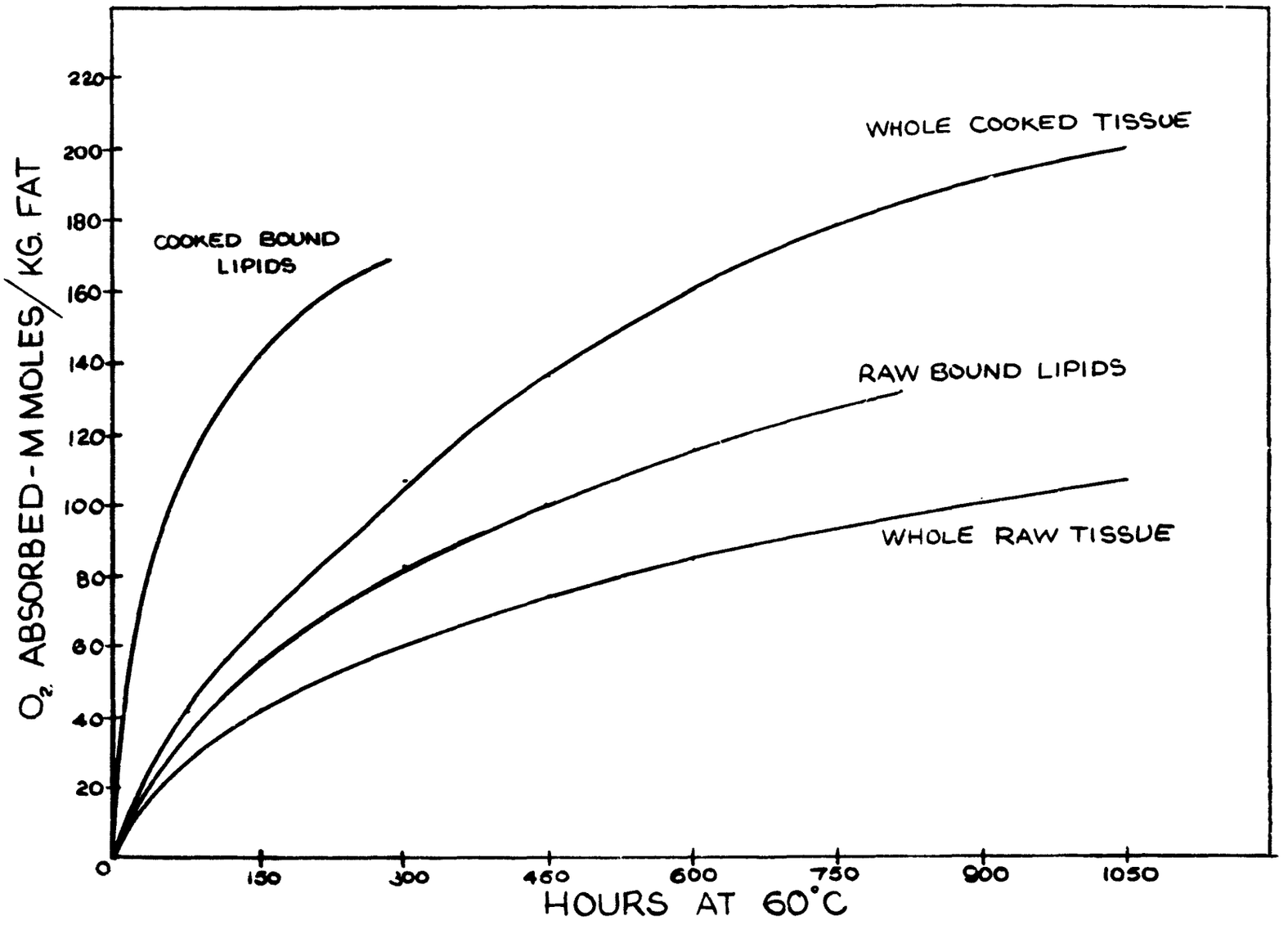

FIG. 14 - OXYGEN ABSORBED BY COOKED AND RAW WHOLE

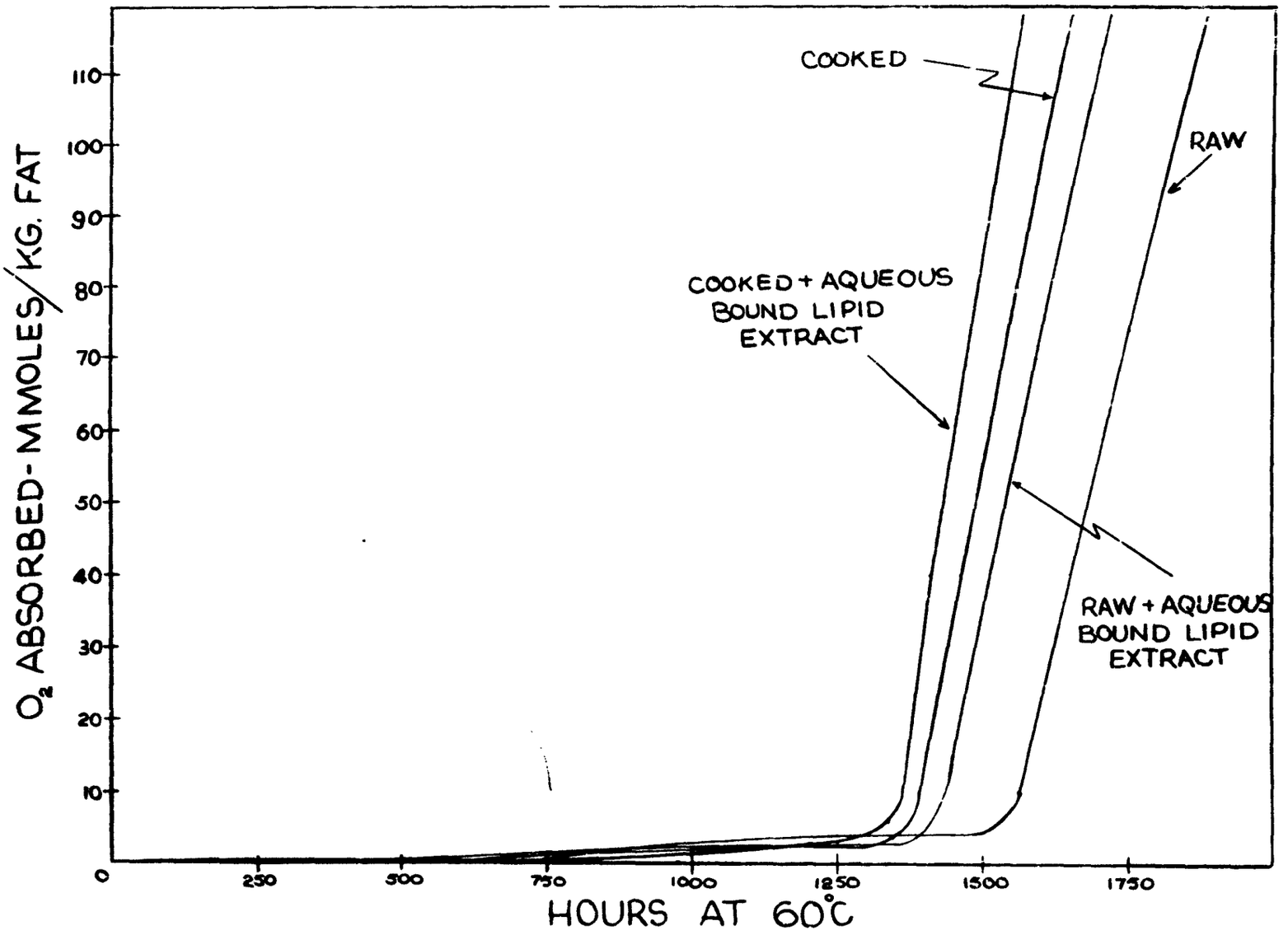

FIG. 15 - OXYGEN ABSORPTION BY GLYCERIDE FATS FROM RAW AND COOKED BEEF RIB EYE SAMPLE NO. 2

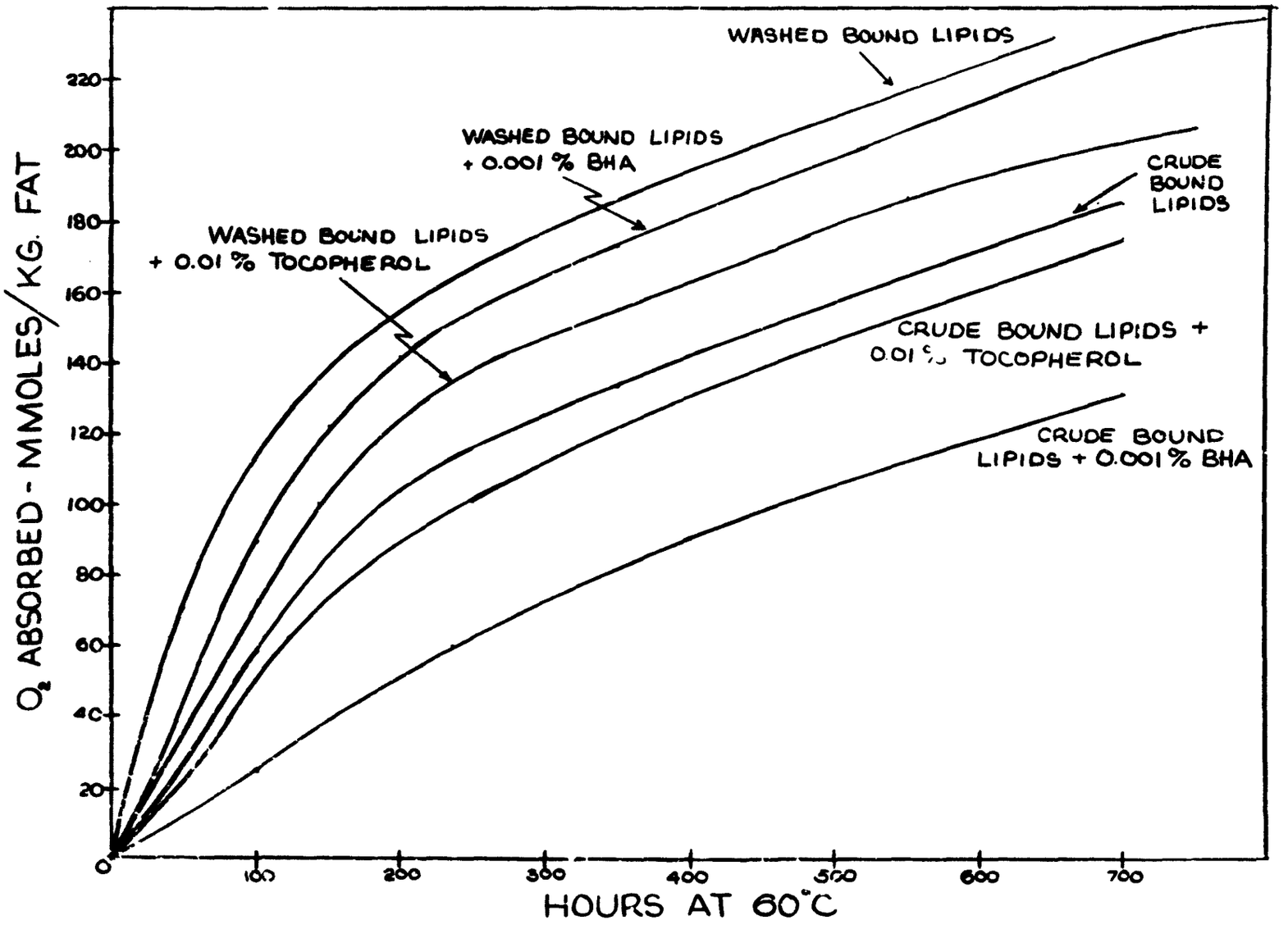

FIG. 16 - EFFECTS OF ANTIOXIDANTS ON RAW BOUND

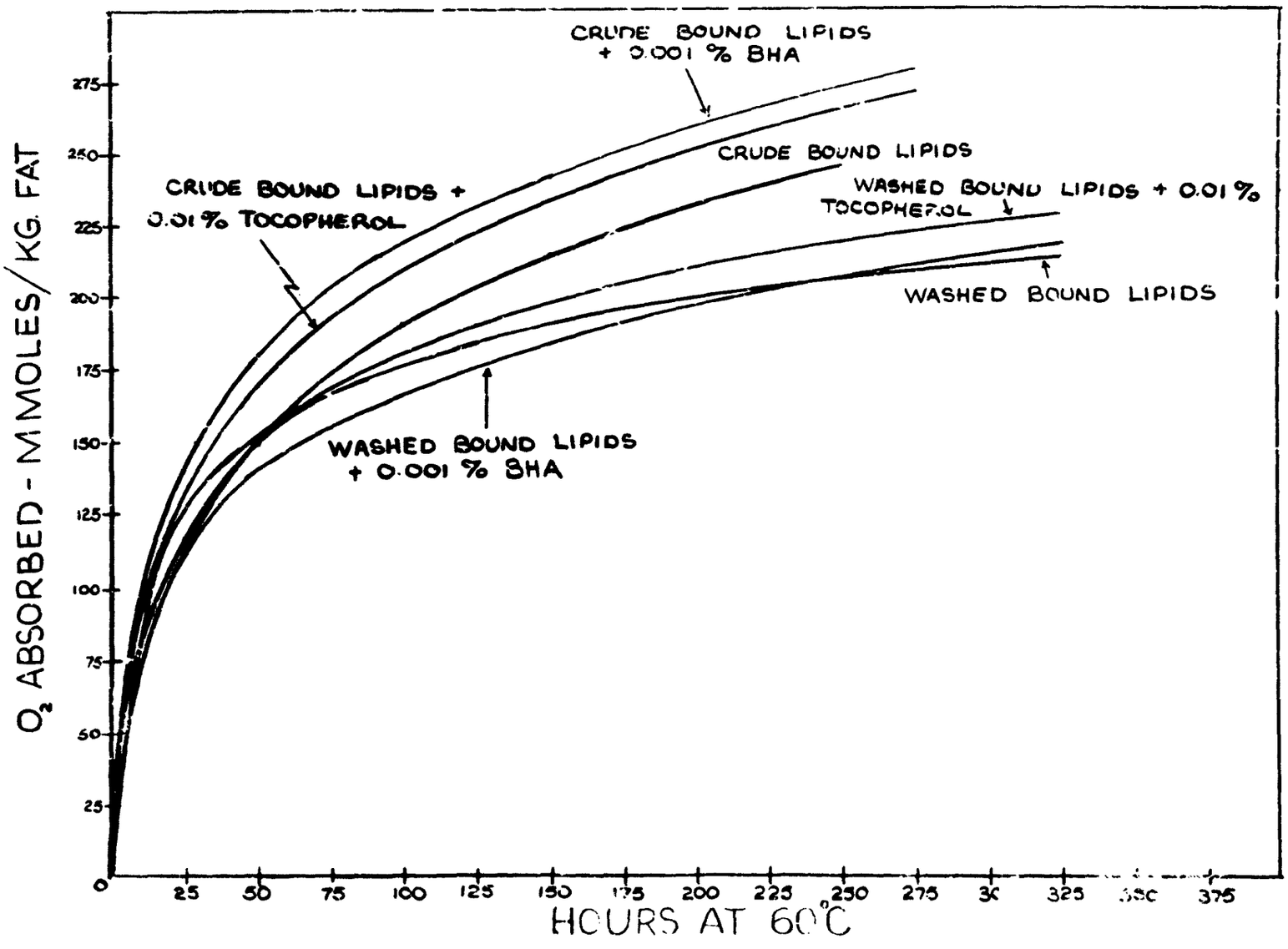

FIG 17 - EFFECT OF ANTIOXIDANTS ON COOKED BOUND LIPIDS

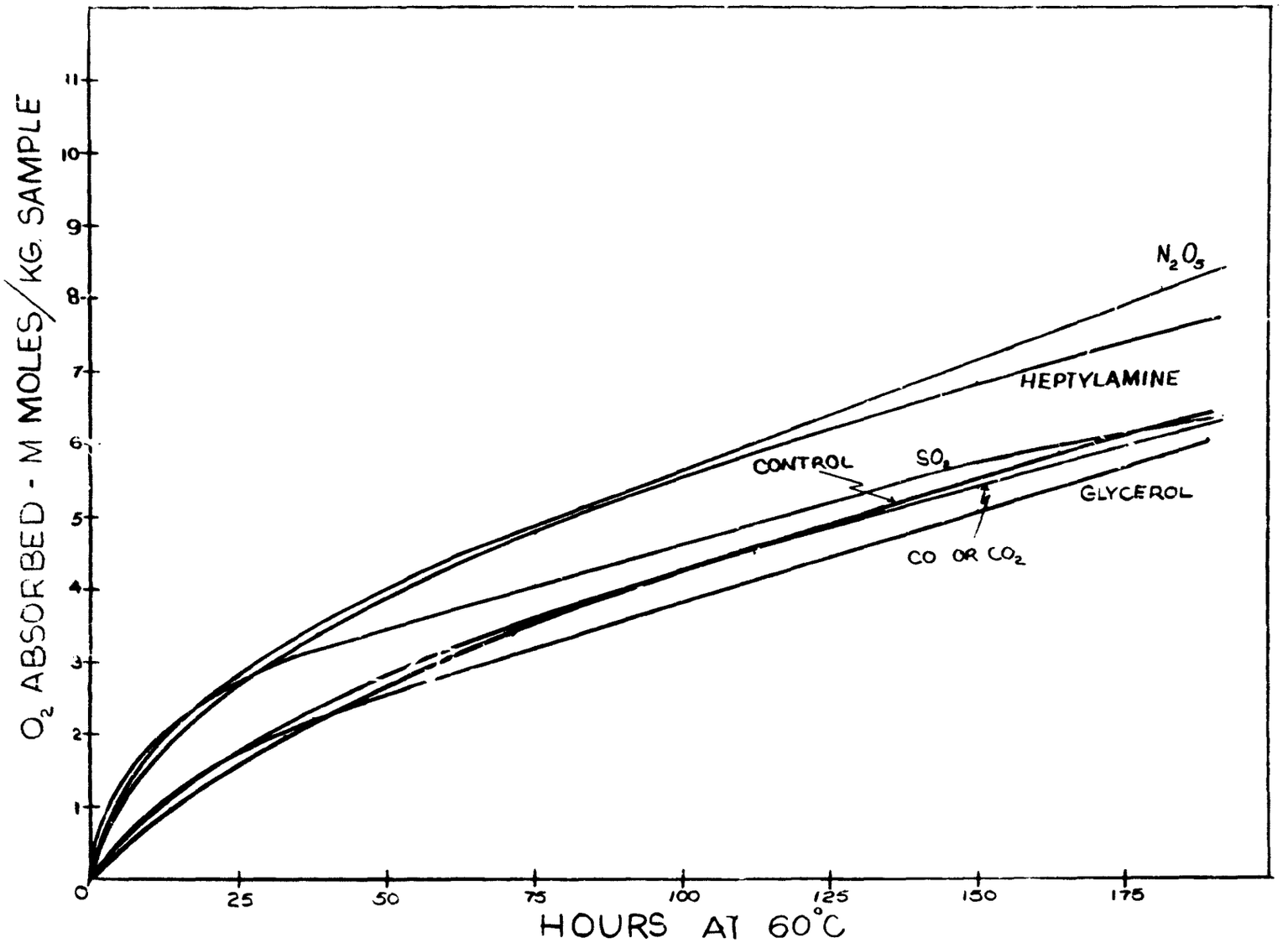

FIG 18 - EFFECT OF POLAR COMPOUNDS ON OXIDATION OF WHOLE FREEZE-DRIED RAW BEEF

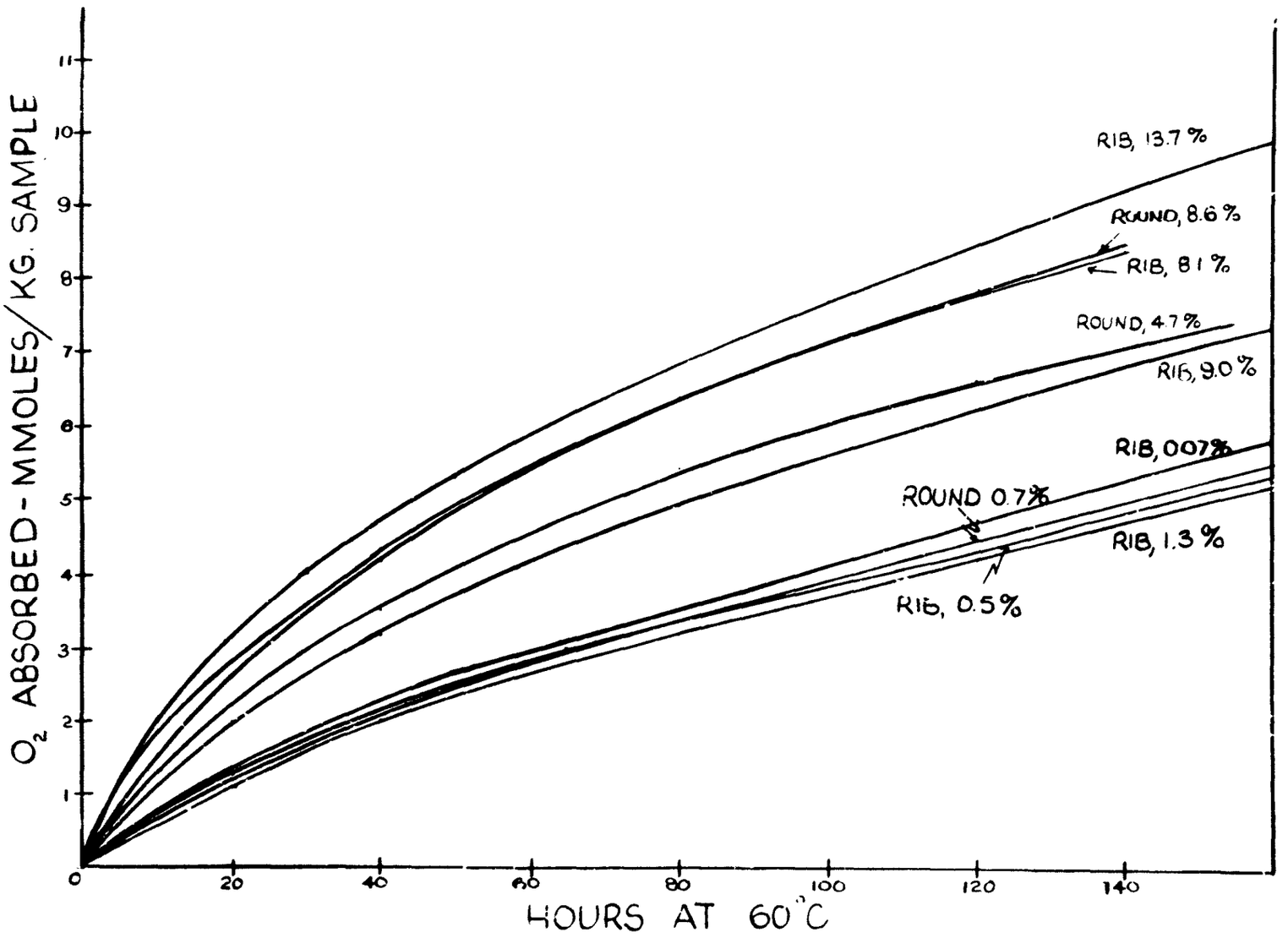

FIG. 19 - EFFECT OF MOISTURE ON OXIDATION OF RIB EYE AND EYE OF THE ROUND FREEZE-DRIED RAW BEEF

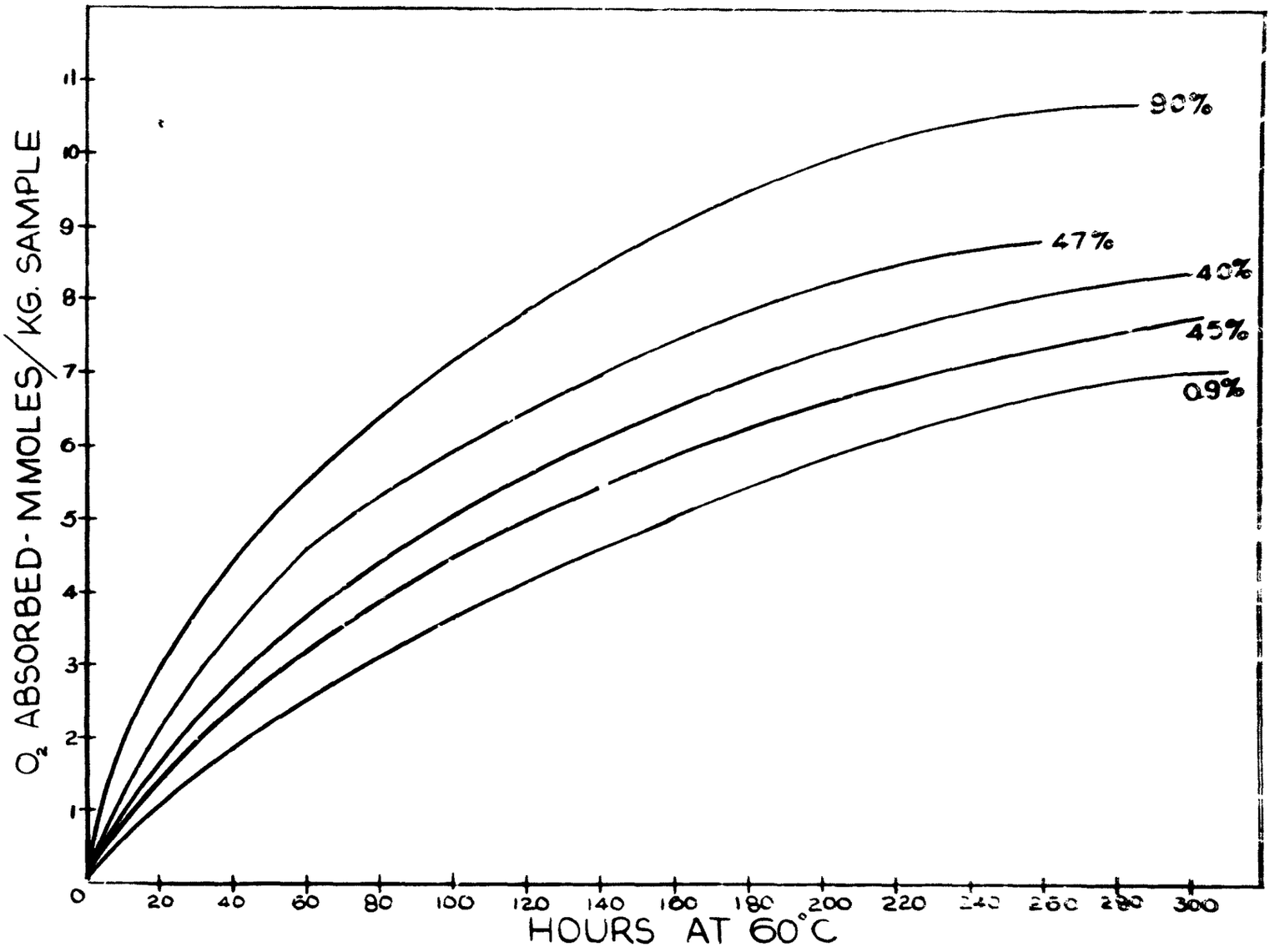

FIG. 20 - EFFECT OF MOISTURE ON OXIDATION OF FREEZE-DRIED RAW RIB EYE NO 2

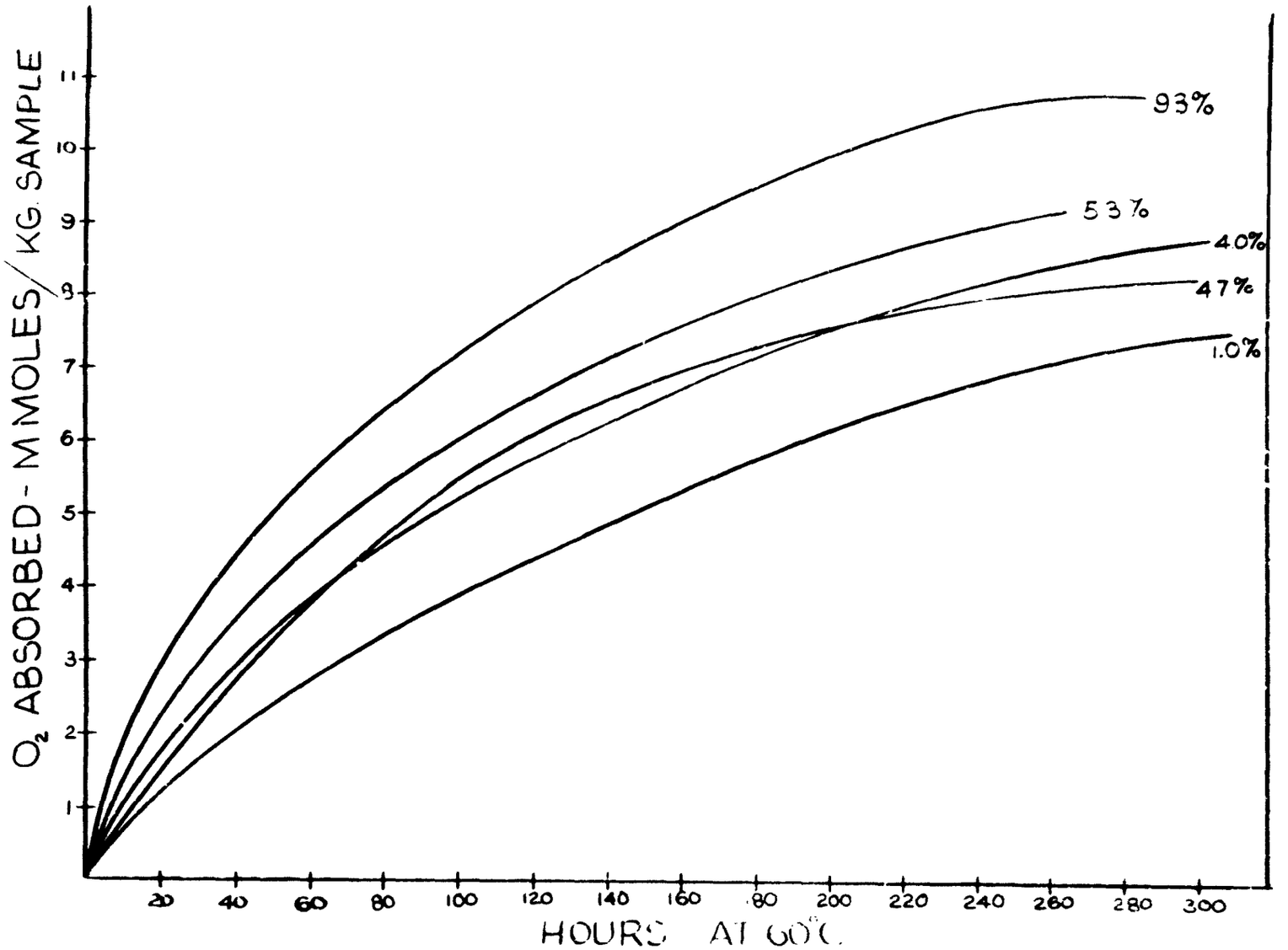

FIG 21 - EFFECT OF MOISTURE ON OXIDATION OF FREEZE - DRIED RAW EYE OF THE ROUND NO. 2